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8 August 1978

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TRANSLATIONS ON USSR SCIENCE AND TECHNOLOGY

BIOMEDICAL AND BEHAVIORAL SCIENCES

No. 42

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No. 42

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CONTENTS (Continued) Page

EPIDEMIOLOGY

UDC: 616.988.75-036.22-02(571.62)"1977"

ETIOLOGICAL AND IMMUNOLOGICAL CHARACTERISTICS OF THE A1 INFLUENZA EPIDEMIC IN KHABAROVSKIY KRAY IN NOVEMBER AND DECEMBER 1977

Moscow VOPROSY VIRUSOLOGII in Russian No 3, 1978 pp 292-297

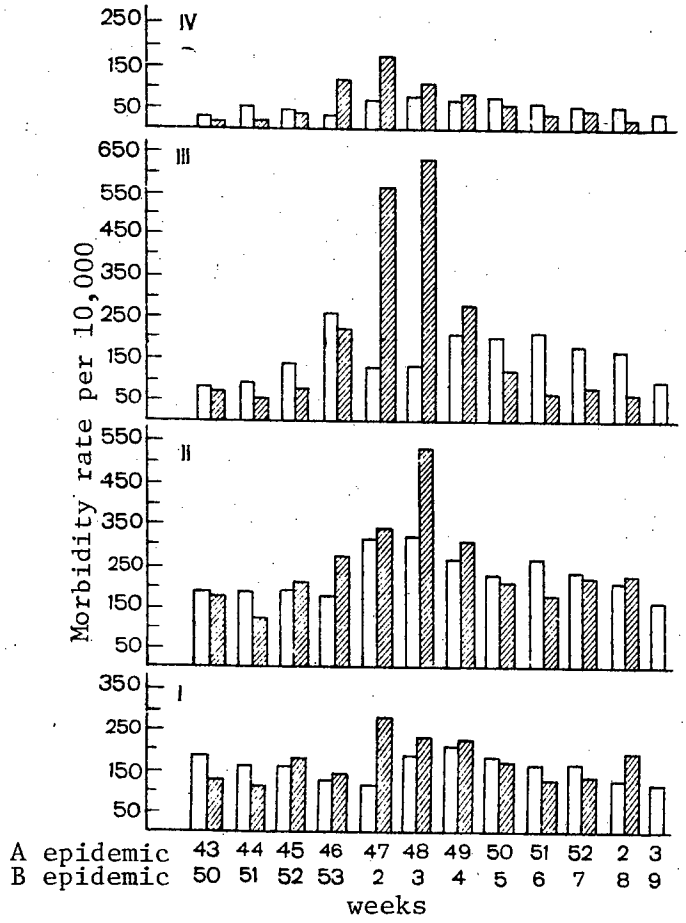
[Article by V. I. Reznik, N. A. Savel'yeva, F. I. Gobomolova, Ye. N. Kirpichnikova, M. A. Pereskokova, A. Ye. Vyborova and N. I. Zdanovskaya, Khabarovskiy Kray Sanitary and Epidemiological Station, submitted 13 Mar 78]

[Text] An epidemic increase in cases of influenza and upper respiratory infections was recorded in Khabarovsk starting on 14 November 1977. There were both sporadic and group cases. The maximum number of sick cases were recorded among individuals up to 22 years of age. Virological studies resulted in isolation of 90 strains of influenza virus during the epidemic; antigenically they were found to be very similar to A1 virus (H1N1) which circulated in our country in 1947-1956. Influenza viruses were isolated only from people under 22 years of age. Serological reactions (IHAR [inhibition of hemagglutination reaction] and CFR [complement fixation reaction]) with paired sera from patients with influenza and demonstration of M immunoglobulins revealed that A1 (H1N1) virus circulated more intensively among individuals up to 22 years of age. A consistency was found between presence of humoral immunity in individuals over 22 years of age and low morbidity rate in these age groups.

In mid November 1977, major outbreaks of acute respiratory diseases (ARD) and clinically severe influenza among students and school children were recorded in Khabarovsk. Several hundred students became sick within 5-7 days at student hostels [dormitories]. The morbidity rate referable to influenza and ARD in that city exceeded the epidemic threshold for 1 week (from 14 to 20 Nov) and the epidemic reached a peak in the next 2 weeks. For the 3 weeks thereafter, the morbidity rate gradually declined and the epidemic was over by 25 December.

The age-related morbidity rate differed from that of previous epidemic outbreaks of influenza, which had been induced by A2 and B viruses. There was

only a 1.9-fold increase in intensive indices at the peak of the epidemic among infants 0 to 3 years of age, as compared to the pre-epidemic level. In the age group of 4 to 7 years, these indices were 3.2 times higher at the peak of the epidemic and 10.3 times higher among children 8 to 14 years of age (see Figure), as compared to the pre-epidemic level. A comparison of age-related morbidity rates during this epidemic and the influenza B epidemic in the fourth quarter of 1976 to first quarter of 1977 revealed that the incidence of influenza was about the same during both epidemics among children up to 3 years od, whereas it was higher during the epidemic of A1 influenza among children 4-14 years of age. After the etiology of the outbreak had been established, one more age group was singled out. A sample was taken of victims of influenza during the period of the epidemic referable to two age groups, 17-21 years and those over 22 years of age, in the area serviced by several polyclinics in the Tsentral'nyy Rayon of Khabarovsk (covering more than 50,000 people). The intensive indices were 6.5 times higher in the former group (3100 sick cases per 10,000 population of the same age) than analogous indices for the older age group (470 per 10,000).



Intensive age-related morbidity indices referable to influenza and ARD during the epidemic of B influenza (4th quarter of 1976--1st quarter of 1977) and A1 influenza (November--December 1977) for Khabarovsk.

Striped columns, A1 flu; white columns, B flu

I) children, 0-3 years old
II) 4-7 years
III) 8-14 years
IV) over 14 years old

Material and Methods

The etiology of the epidemic was identified by virological and serological methods. Influenza virus was isolated on chick embryos. An inoculation and two passages were performed. The first nasopharyngeal specimens were taken from students during epidemic outbreaks. Subsequently, the material was taken from both hospitalized patients and therapeutic, as well as pediatric districts in different parts of the city.

The method of end dilutions and titrations in the presence of guinea pig serum was used to obtain inhibitor-resistant variants of influenza virus. The obtained inhibitor-resistant variant was used to prepare diagnosticum, with which serum from patients and donors was tested. In addition, we used for IHAR commercial diagnosticums A1, A0, A/Port Chalmers/1/73, A/Hong Kong/68, B, parainfluenza types 1 and 2; for the CFR, we used diagnosticums A1, B, antigens of adenoviruses and respiratory syncytial (RS) virus. The cysteine test was used for demonstration of IgM.

Results and Discussion

In all, we submitted 268 patients to virological testing during the epidemic period; 90 (33.6%) strains of influenza virus were isolated from them. The viruses were isolated both from the first inoculation (23.3%) and first passage (43.3%), as well as second passage (33.3% of all strains isolated). More of the material taken from students in flu sites contained virus than from patients in household sites. Virus carriers constituted 53.3 to 64.5% in student dormitories [hostels], 11.1 to 38.5% in the pediatric and therapeutic districts.

Table 1. Results of virological screening of patients with ARD and flu during the outbreak of A1 flu in Khabarovsk, 23 Nov to 26 Dec 77

Age group	Number of subjects	Flu virus isolated	
		absolute	%
0—3 years	57	10	17,5
4—7 "	38	6	15,7
8—14 "	63	20	31,7
15—22 "	104	54	51,9
23 yrs and older	6	0	0
totals	268	90	33,6

Individuals of different ages were submitted to virological screening. Viruses were isolated at about the same incidence from children 0-3 and 4-7 years old: 17.5 and 15.7%, respectively. In school children through 14 years of age, this index was higher (31.7%), and it constituted 51.9% in the group 15-22 years old. We could not isolate the virus from individuals over 22 years old, although there were few such cases (Table 1).

Table 2. Sensitivity to normal animal serum inhibitors of A influenza virus strains isolated in Khabarovsk in November and December 1977

	Number of virus strains	Number of strains reacting in IHAR to titer indicated								
		native serum			serum heated to 58°C for 30 min			rivanol-treated serum		
		0—16	32—64	128—512	0—16	32—64	128—512	0—16	32—64	128—512
Serum from										
guinea pig	55	3	41	11	38	16	1	55	0	0
rabbit	55	0	5	50	0	30	25	54	1	0
horse	55	50	5	0	54	1	0	55	0	0

Table 3. Results of laboratory identification of influenza and ARD cases in Khabarovsk in Nov-Dec 77 during outbreak of A1 flu (data of virological laboratory of Kray Sanitary and Epidemiological Station)

Date	Virological			Sum of virological and serological methods									
	total exam.	virus isolated		total exam.	cases of A flu isolated		total cases of A flu*		B flu	RS virus	adeno-virus	para-influ-enza	mixed infec-tions
		abs.	%		A flu	including A1	abs.	%					
31/X—6/XI	—	—	—	10	2	—	3	30,0	1	1	—	—	—
7/XI—13/XI	—	—	—	7	3	2	3	42,9	—	—	—	—	—
14/XI—20/XI	11	5	45,5	39	26	15	27	69,2	—	—	—	1	1
21/XI—27/XI	74	38	51,3	100	61	46	68	68,0	1	—	1	1	6
28/XI—4/XII	94	30	31,9	130	41	33	42	32,3	—	2	—	—	1
5/XII—11/XII	23	7	30,4	42	13	8	16	38,1	—	—	—	—	3
12/XII—18/XII	29	8	27,6	86	22	15	27	31,4	—	—	7	—	5
19/XII—25/XII	29	1	3,4	52	8	5	9	17,3	1	—	6	1	—
26/XII—1/I	8	1	12,5	38	6	3	8	21,0	1	—	1	—	2
totals	268	90	33,6	504	182	127	203	40,3	4	3	15	3	19

*Total number of confirmed cases of influenza A, including combinations thereof with other infections.

Note: Processing done according to date of illness.

The dynamics of flu cases with positive isolation of virus indicate that the virus had circulated in Khabarovsk already since 14 November. For the first 2 weeks of the outbreak, the incidence of virus isolation constituted 45.5 and 51.3%, and about 30% for the next 3 weeks. The last 2 strains were isolated from individuals who became sick on 24 and 26 December. The strains were typed using the IHAR with specific sera to influenza A(HONl), A(HlNl), A(H3N2), B (produced by the Leningrad Scientific Research Institute of Vaccines and Sera), as well as A/Victoria/3/73(H3N2) and A/New Jersey/8/76 (HswlNl) viruses, obtained from the All-Union Scientific Research Institute of Influenza. All 90 strains interacted with A(HlNl) serum, and 60% did so to a complete serum titer, while the rest did so to one-half the titer. A total of 51 strains was tested with an expanded set of specific sera; 18 of these strains presented retention in the IHAR with serum A(HONl) to one-thirty-second of the titer, in addition to serum A(HlNl). Immune serum to the two strains of isolated virus was obtained by immunizing roosters. This serum was tested in the IHAR with a set of antigens of A(HONl), A(HlNl), A/Port Chalmers/1/73(H3N2), A/Hong Kong/68(H3N2) and A/Khabarovsk/34561/77 (HlNl). Retarded hemagglutination was demonstrated with homologous A/Khabarovsk/34561/77 virus to a titer of 1/512 (to a titer of 1/64 after rivanol treatment(and with A(HlNl) virus to a titer of 1/256 (to a titer of 1/32 after rivanol treatment). The new virus may be considered antigenically very similar to A(HlNl) virus, which circulated up to 1957. Sensitivity to antihemagglutinating inhibitors of normal guinea pig, rabbit and horse serum was tested in 55 strains (Table 2). Most strains presented little sensitivity to inhibitors of horse serum, they were more sensitive to inhibitors of guinea pig serum, and virtually all were highly sensitive to inhibitors of native rabbit serum. Heating to 58°C for 30 min attenuated inhibition for most strains with all types of serum, while rivanol treatment eliminated it completely.

Table 4. Immunological characteristics of different age groups of residents of Khabarovsk with respect to A(HlNl) virus prior to the epidemic in November and December 1977

Age group	Number of cases	Geometric mean of titer	% cases with antibodies	
			⩽1/8	⩽1/16
0—7 years	57	1:21,1	33,3	64,9
8—22 "	83	1:19,1	26,7	58,3
⩾23 "	67	1:35,5	5,2	26,7

During the period of the outbreak, from 14 November 1977 to 1 January 1978, a total of 291 patients were submitted to serological examination; diagnostic seroconversion to influenza A virus (overall, according to CFR and IHAR) were demonstrated in 48.1%. The incidence of seroconversions was not the same in different age groups of children: 28.2% positive reactions (39 children tested) among children 0 to 14 years of age, 59.1% in individuals

Table 5. Comparative intensity of immunological changes in individuals who contracted (clinically) A1 influenza in November and December 1977, in Khabarovsk (from 14 November to 26 December 1977)

Age group	Antigens	A1 standard	A1/Khabarovsk/77	A0/Shklyaver/48	A2/Hong Kong/68	A2/Port Chalmers/68	A/New Jersey/8/76
Up to 21 years	Patients tested	201	192	183	201	201	133
	% with 4-fold or greater antibody increment	14,4	18,2	15,3	1,5	0,5	4,5
	geometric mean titer serum I	1:22,6	1:60,0	1:12,1	1:84,0	1:52,0	1:30,0
	serum II	1:30,0	1:79,0	1:18,4	1:79,0	1:49,0	1:28,0
	T	2,8	2,5	2,7	<1	<1	<1
22 years and older	Patients tested	61	61	61	61	61	
	% with 4-fold or greater antibody increment	6,6	9,8	11,5	1,6	3,3	
	geometric mean titer serum I	1:21,1	1:34,0	1:16,0	1:69,0	1:30,0	
	serum II	1:24,2	1:64,0	1:22,6	1:64,0	1:28,0	
	T	1,0	4,0	1,8	<1	<1	

15 to 21 years of age (181 tested) and 30.9% among those 22 years of age or older (71 tested).

The overall findings of laboratory tests (virological and serological) indicate that there was laboratory confirmation of A influenza in 69.2-68.0% of the patients in the first 2 weeks of the outbreak, for the next 3 weeks this index was slightly above 30% and it was about 20% for the last 2 weeks of December (Table 3).

When the pathogen of the epidemic was identified, analysis was made for demonstration of hemagglutinins to A1 virus in blood serum taken from individuals in different age groups in the second and third quarters of 1977 (Table 4). Before the epidemic, individuals up to 22 years of age were found to be the least protected; antibody titers did not exceed a 1/16 dilution in 64.9-58.3% of these individuals. At the same time, among those over 23 years of age, only 26.7% presented such low antibody titers and only 5.2% presented an antibody level of 1/8. A comparative study of intensity of immunological changes in patients suffering from influenza, as related to different types of A influenza virus, revealed that most often there was a diagnostic increment of antihemagglutinins to A/Khabarovsk/77 and A(H1N1)--standard diagnosticum--viruses (Table 5). In the group up to 21 years old, positive reactions were found twice as often as in those over 22 years of age. In some of the subjects, there was a diagnostic elevation of titer of antbodies to A(HONl) influenza virus. This circumstance, as well as the results of studying the type structure of the isolated virus, are indicative of some relationship between the new virus and A(HONl) virus.

We screened patients and donors to demonstrate IgM. IgM was found more often among patients up to 21 years of age than those over 22 years old (Table 6). Since the presence of IgM is indicative of the first encounters of the body with antigen, demonstration thereof in a high percentage of young individuals is indicative of the novelty of this virus to them and immunity to this virus in the older age groups. We tested donor blood serum taken in December 1977, i.e., during the outbreak of influenza. IgM to A/Khabarovsk/77 virus was found in 23.5% of 34 individuals up to 21 years of age and in 15.6% of 64 donors over 22 years old. Since these were healthy individuals, demonstration of IgM is indicative of asymptomatic prior infection.

Table 6. Incidence of IgM in blood serum of patients suffering from influenza in November and December 1977

Age group	Number of patients tested	Individuals with IgM to A1 virus (standard diagnosticum), %
0-3 years	52	19.2
4-14 years	50	26.0
15-21 years	96	28.1
22 years and older	93	9.7

A comparison of the epidemic data and results of laboratory studies of the epidemic suggests that the unique age-related incidence of influenza is related primarily to the existence of type-specific immunity to A1(H1N1) virus among individuals who had encountered the virus 22 years previously. Evidently, the duration of humoral immunity to A1(H1N1) influenza virus, which had a substantial influence on morbidity, constitutes at least 20 years.

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EPIDEMIOLOGY

UDC 616.988.75-036.22-037(47+57)

PREDICTION OF INFLUENZA EPIDEMICS IN THE CONDITIONS OF THE USSR

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 131-137

[Article by O. V. Baroyan and L. A Rvachev, Institute Epidemiology and Microbiology imeni I. F. Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] As we know influenza is the most widespread infectious disease. This infection surpasses all others taken together in relation to the number of people afflicted. The scale of its incidence can be deduced just from the fact that at times of some epidemics in Western Europe, up to 1 million persons have been unable to work and that the total number of striken persons was around 10 million. Influenza epidemics recur systematically every 1-3 years, and about once every 5-10 years they grow into pandemics during which a fourth of all mankind contracts the disease.

Not only is it important in this connection to make a qualitative prediction of influenza epidemics--will there be an epidemic or not?; a quantitative prediction of the course of epidemics, especially prediction of the days of "peak" situations in different cities, is much more important. On being sent to interested organizations, such a prediction would permit us to create certain resources beforehand and plan the distribution of manpower and resources along both economic and medical lines.

In order to predict the numerical course of influenza epidemics we must have a mathematical model of these epidemics for the territory of the country under examination. Let us imagine a country to be a sum of n cities and, as desired, other geographical elements; the selection of this geographical structure is quite arbitrary , and it depends on the interest of the public health agencies. Let us number the cities from 1 to n; the subscript i means that the parameter value pertains to the city number i. Next we adopt the following symbols; $x_i(t)$-- the number of susceptible persons at time t; $y_i(\tau,t)$-- distribution, with respect to τ at time t, of those persons for whom time τ had passed since the moment of their infection; λ-- mean frequency of infection transmission; p_i--population; $g(\tau)$-- the probability of being a carrier of infection at time τ after infection; T-- maximum length of the contagious period; σ_{ij}-- passenger turnover intensity between elements i and j; α--initial density of susceptible persons. Then the equation of the influenza epidemic model would assume the following form;

$$\frac{dx_i}{dt} = -\frac{\lambda}{p_i} x_i \int_0^T y_i(\tau, t)\, g(\tau)\, d\tau + \sum_{j=1}^{n} \left(\frac{\sigma_{ji}}{p_j} x_j - \frac{\sigma_{ij}}{p_i} x_i \right);$$

$$\frac{\partial y_i}{\partial t} + \frac{\partial y_i}{\partial \tau} = \sum_{j=1}^{n} \left(\frac{\sigma_{ji}}{p_j} y_j - \frac{\sigma_{ij}}{p_i} y_i \right);$$

$$y_i(0, t) = \frac{\lambda}{p_i} x_i(t) \int_0^T y_i(\tau, t)\, g(\tau)\, d\tau;$$

$$x_i(0) = \alpha p_i,\ i = 1, 2, \ldots, n,$$
$$y_i(\tau, 0) \text{ at } 0 \leqslant \tau \leqslant T.$$

There are also certain algorithms for computing the parameters and the initial state of this system of equations and, moreover, there are methods for modeling recorded morbidity (that is, visits to physicians) using this system; this morbidity differs from true morbidity for two reasons: a) Not all patients visit physicians; b) the frequency of visits depends on the day of the week, which means that there are weekly cycles in recorded morbidity. The model's geographical elements for the USSR included one hundred cities with the largest populations and 28 selected regions embracing practically the entire population of the country (Figure 1). In addition the model's elements include data on daily passenger turnover between USSR cities and regions, the total for trains, airplanes, and buses (Figure 2).

Let us summarize the prediction for the USSR.

First we introduce the prediction assessment criterion. Obviously we must base ourselves not on the standard ideas of mathematical statistics but rather on the requirements of the "client"--that is, the public health and economic agencies. What is most important to them is prediction of the beginning of the epidemic in each city, and especially the time of the peak of the outbreak. If this day is predicted with an accuracy within one week, the prediction can be assumed to be satisfactory, all the more so because the the computer specifies the place of the entire epidemic wave of the given city on the time axis. We will refer to the requirement for predicting the times of outbreak peaks in the cities with this accuracy as the "zone" criterion (the meaning of this term will be clarified below).

It is important for practicing agencies to known not only the time but also the intensity of the outbreak, and especially the height of its peak-- that is, the morbidity that will be recorded during the "most difficult day." Let us treat the following requirements as the "square" criterion; the moment of the outbreak peak must be predicted with an accuracy of up to 5 days (that is, the requirement imposed on the "zone" is reinforced by this requirement) and concurrently, the prediction of the height of this peak must not deviate from the actual height by more than 1.5 times (that is, it must be from 67 to 149 percent of the actual height).

The meaning and order of use of these criteria are as follows. We designate the relationship between the computed (that is, predicted) height of the

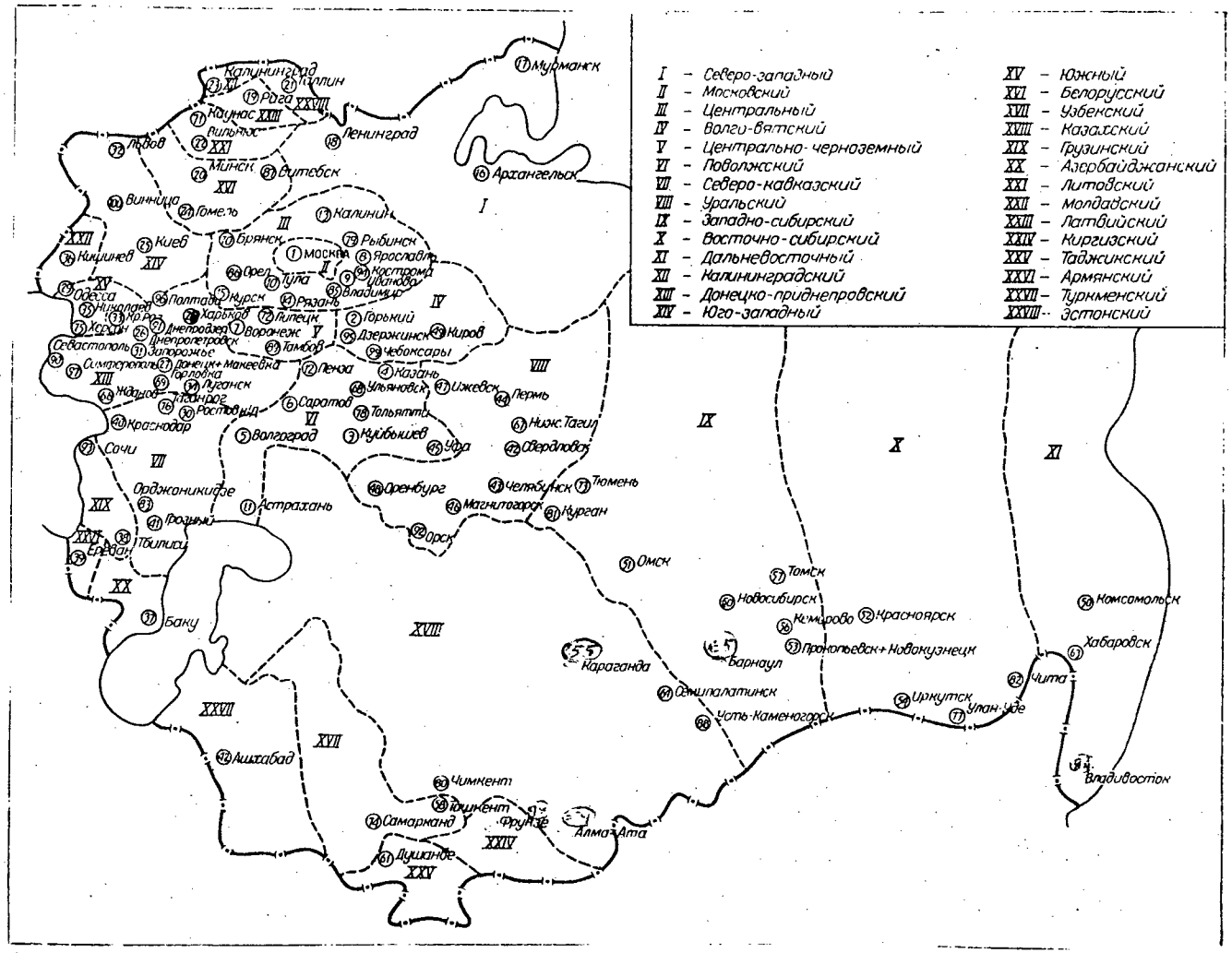


Figure 1. Selected regions of the USSR

[Key on following page]

Key:

1. Moscow
2. Gor'kiy
3. Kuybyshev
4. Kazan'
5. Volgograd
6. Saratov
7. Voronezh
8. Yaroslavl'
9. Ivanovo
10. Tula
11. Astrakhan'
12. Penza
13. Kalinin
14. Ryazan'
15. Kursk
16. Arkhangel'sk
17. Murmansk
18. Leningrad
19. Riga
20. Minsk
21. Tallin
22. Vil'nyus
23. Kaliningrad
24. Gomel'
25. Kiev
26. Dnepropetrovsk
27. Donetsk + Makeyevka
28. Khar'kov
29. Odessa
30. Rostov-on-Don
31. Zaporozh'ye
32. L'vov
33. Krivoy Rog
34 Lugansk
35. Nikolayev
36. Kishinev
37. Baku
38. Tbilisi
39. Yerevan
40. Krasnodar
41. Groznyy
42. Ashkhabad (Sverdlovsk)
43. Chelyabinsk
44. Perm'
45. Ufa
46. Magnitogorsk
47. Izhevsk
48. Orenburg
49. Kirov
50. Komsomol'sk
51. Omsk
52. Krasnoyarsk
53. Prokop'yevsk + Novokuznetsk
54. Irkutsk
55. Karaganda
56. Kemerovo
57. Tomsk
58. Tashkent
59. Alma-Ata
60. Novosibirsk
61. Dushanbe
62. Karaganda
63. Khabarovsk
64. Semipalatinsk
65. Barnaul
66. Zhdanov
67. Nizhniy Tagil
68. Ul'yanovsk
69. Gorlovka
70. Bryansk
71. Kaunas
72. Lipetsk
73. Tyumen'
74. Samarkand
75. Kherson
76. Taganrog
77. Ulan-Ude
78. Tol'yatti
79. Rybinsk
80. Chimkent
81. Kurgan
82. Chita
83. Ordzhonikidze
84. Vladivostok
85. Vladimir
86. Orel
87. Vitebsk
88. Ust'-Kamenogorsk
89. Tambov
90. Sevastopol'
91. Dneprodzerzhinsk
92. Orsk
93. Sochi
94. Kostroma
95. Dzerzhinsk
96. Poltava
97. Simferopol'
98. Frunze
99. Cheboksary
100. Vinnitsa

I Northwestern
II Moscow
III Central
IV Volga-Vyatka
V Central Chernozem
VI Povolzh'ye
VII Northern Caucasian
VIII Ural
IX Northern Siberian
X Eastern Siberian
XI Far Eastern
XII Kaliningrad
XIII Donetsk-Pridneprovsk
XIV Southwestern
XV Southern
XVI Belorussian
XVII Uzbek
XVIII Kazakh
XIX Georgian
XX Azerbaydzhan
XXI Lithuanian
XXII Moldavian
XXIII Latvian
XXIV Kirgiz
XXV Tadzhik
XXVI Armenian
XXVII Turkmen
XXVIII Estonian

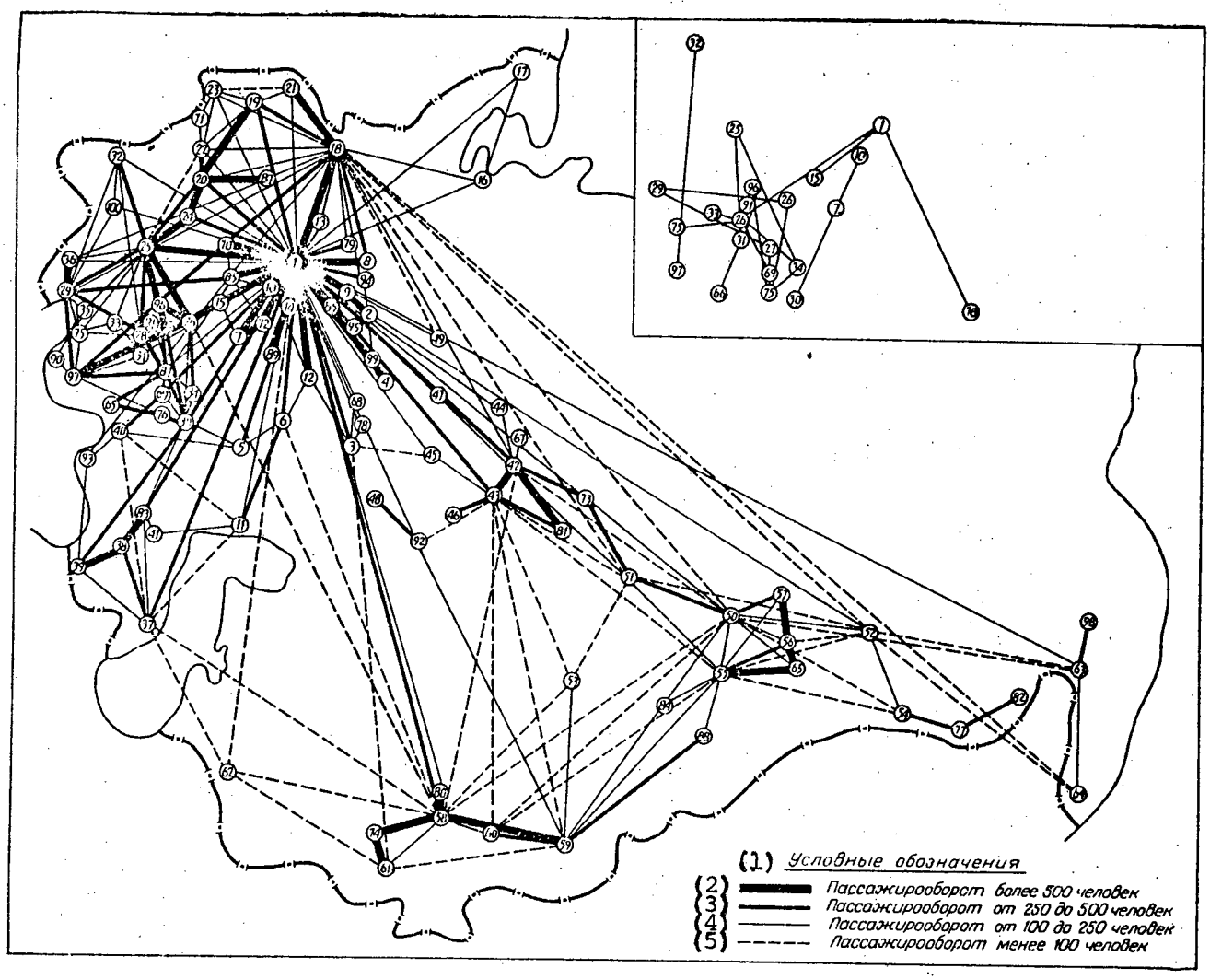


Figure 2. Daily passenger turnover

Key:

1. Symbols
2. Passenger turnover greater than 500 persons
3. Passenger turnover from 250 to 500 persons
4. Passenger turnover from 100 to 250 persons
5. Passenger turnover less than 100 persons

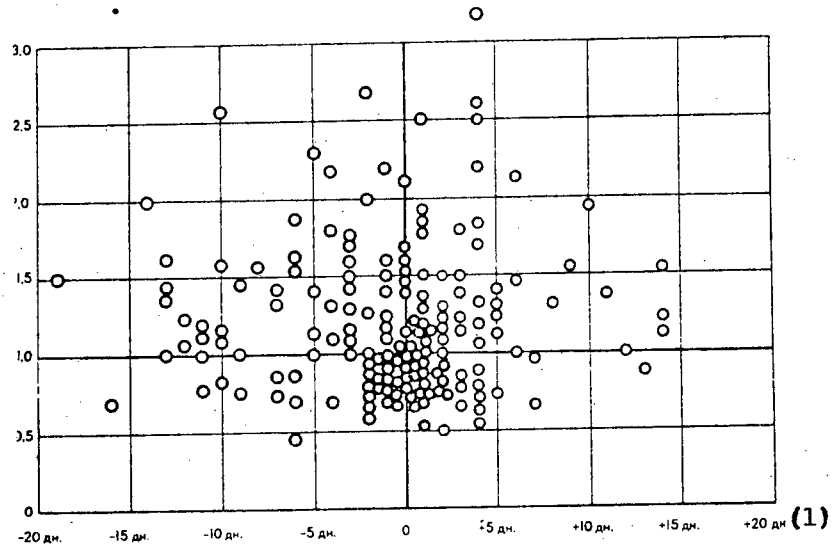


Figure 3. Total prediction results for the epidemics of 1957, 1959, 1962, 1965, 1967, 1969, 1970 (170 outbreaks in USSR cities are shown)

Key:

1. Days

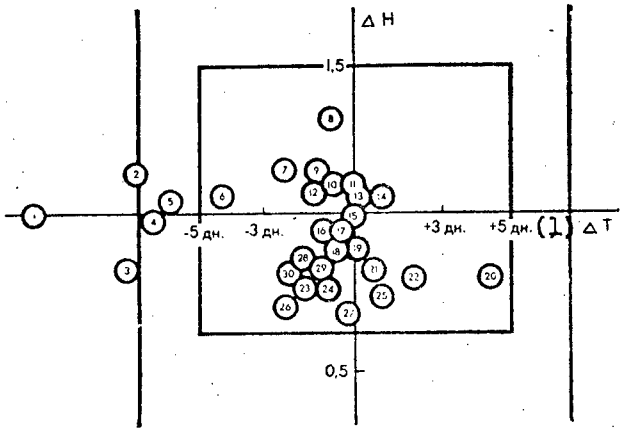


Figure 4. Results of predicting the epidemics of 1971-1972

Key:

1. Days

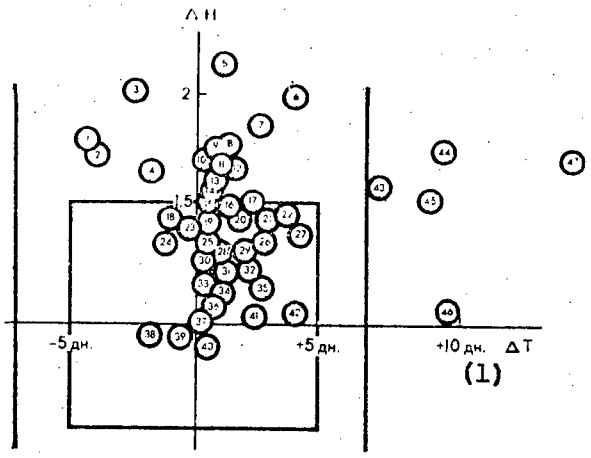
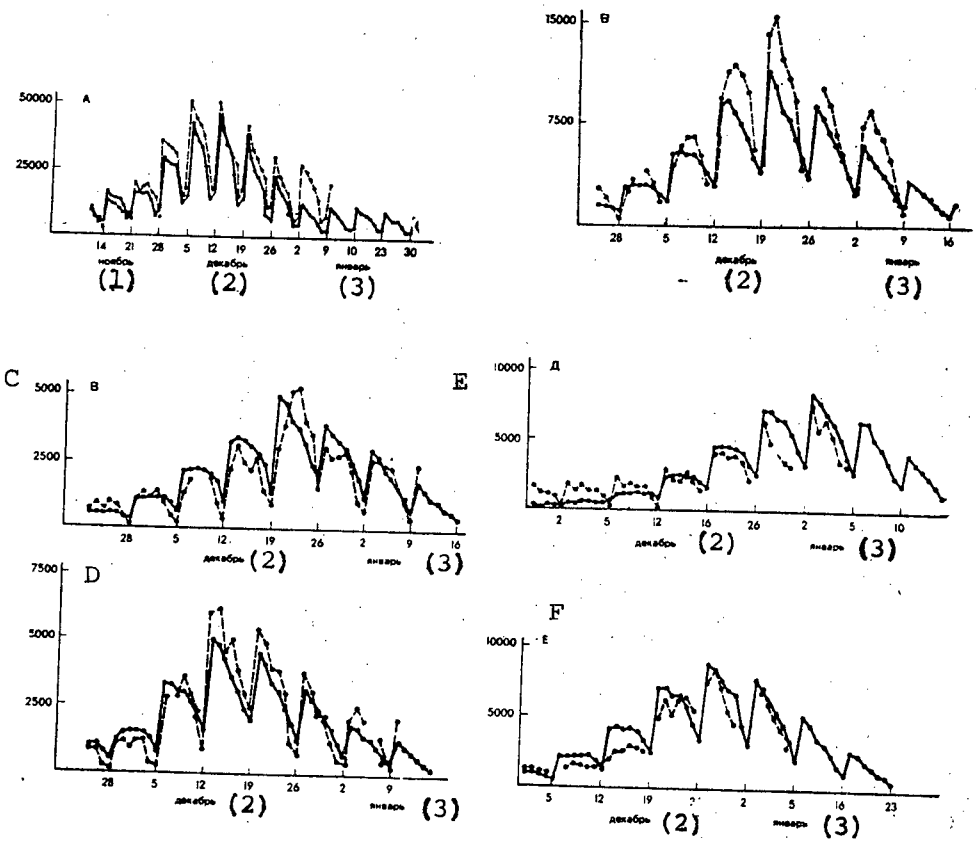


Figure 5. Results of predicting the epidemics of 1973

Key:

1. Days



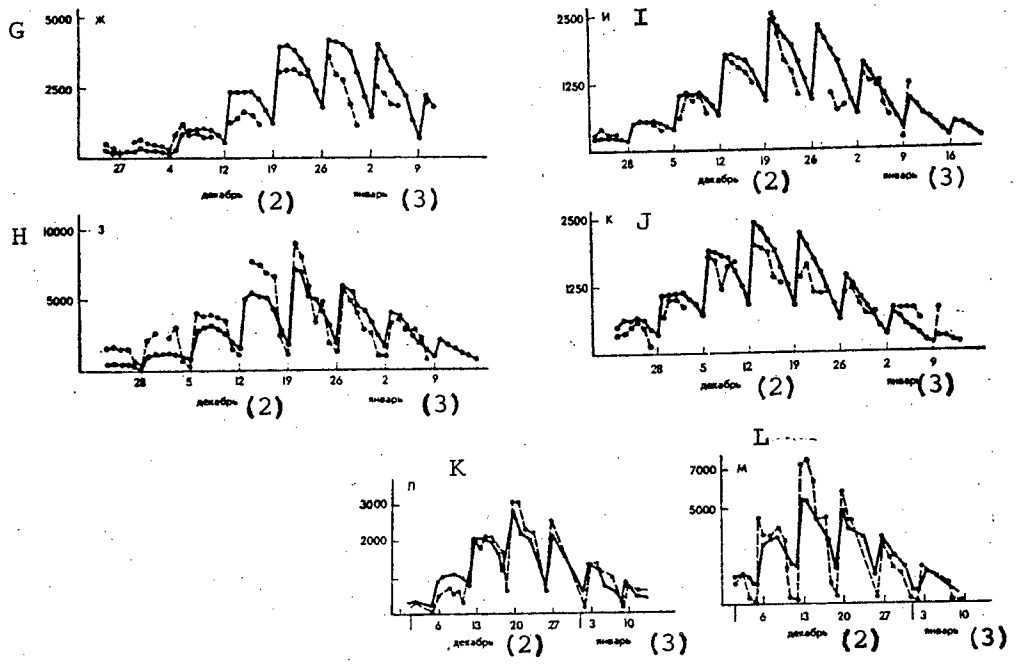


Figure 6. Examples of Using the Model in Public Health Practice: Prediction of influenza epidemics in USSR cities in 1971-1972. The broken line (A-J) and the dots (L,M) are actual daily morbidity (gaps indicate that data for the given date are absent); the solid line is the computer prediction. A--Moscow; B--Kiev; C--Riga; D--Alma-Ata; E--Baku; F--Tashkent; G--Yerevan; H--Minsk; I--Vil'nyus; J--Tallin; K--Frunze; L--Tbilisi

Key:

1. November
2. December
3. January

morbidity peak for city i and the actual height as ΔH_i, and the difference between the computed day for the peak and the actual day as ΔT_i. Thus for an ideal prediction, $\Delta H_i=1$, $\Delta T_i=0$. There is certain interest in assessing the results of the prediction in relation to each characteristic ΔH, ΔT taken seperately (as had been noted earlier, ΔT_i is especially important to practicing agencies, but it does not reveal the nature of the situation, since while the height of the peak might be predicted well, its time might be predicted poorly, and vice versa). Moreover we must make an average assessment of the epidemic prediction for the country as a whole, since a poor prediction for specific cities is unimportant, inasmuch as we have no guarantee against large fluctuations. The following graphical method for total assessment of the "national" epidemic prediction based on both characteristics ΔT_i and ΔH_i and in relation to all cities concurrently appears to be the most descriptive and exhaustive; representation of outbreaks in each city as circles on the coordinate plane ΔT, ΔH at a point having coordinates equal to the errors of ΔT_i and ΔH_i in the prediction of this

outbreak. In this case the computer prediction can be interpreted as sighting "fire" at a point with coordinates 0.1 (representing an ideal prediction), and the quality of this "fire" can be assessed immediately from the corresponding ellipse of scatter on plane ΔT, ΔH (3-5). With ideal "fire," the figures would have only one circle with coordinates 0.1, since all "shells" would strike that point. In view of inevitable prediction errors, a certain amount of scattering occurs, but we can see from Figures 3-5 that the concept of predicting influenza epidemics using this model is justified, since we can observe significant grouping of the circles about the ideal prediction. Were we to superimpose a coordinate type of grid over plane ΔT,ΔH, then we could use the number of circles falling within particular squares as the basis for making a quantitative assessment of prediction quality, this assessment taking the form of the corresponding probability distributions. In particular, the "zone" and "square" criteria are emphasized in Figures 3-5 by heavy lines.

In these terms, the results of the prediction for the USSR are as follows:

a) Figure 3 shows the results of a major test of the model based on all available information for the epidemics of 1957-1970; the "zone" criterion is satisfied by the prediction for 80 percent of all outbreaks (the test was performed with information concerning 170 outbreaks), while the "square" criterion is satisfied for 55 percent of all outbreaks;

b) Figure 4 shows the results of a prediction made of the 1971-1972 epidemic by the All-Union Scientific Research Institute of Influenza; the prediction satisfies the "zone" criterion for 90 percent of all outbreaks, and it satisfies the "square" criterion for 80 percent of all outbreaks (the Figures 4 and 5 were provided to us by Yu. G. Ivannikov, laboratory director, All-Union Scientific Research Institure of Influenza, and they are published with his permission);

c) the results of the prediction made by the All-Union Scientific Research Institute of Influenza for the 1973 epidemic are shown in Figure 5; the prediction satisfies the "zone" criterion for 96 percent of all outbreaks, and it satisfies the "square" criterion for 78 percent of all outbreaks.

Similar results were obtained in predictions of the 1975 and 1976 epidemics.

Thus judging from all influenza epidemics beginning in 1957, we can say that the prediction capabilities of the USSR model are fully satisfactory.

The reader can gain a deeper acquaintance with the nature of computer prediction from Figure 6, showing examples of predictions for the 1971-1972 epidemics (in the capitals of the union republics).

Thus a model predicting the quantitative course of influenza epidemics has been built for the USSR territory, and major tests of this model have demonstrated the satisfactory nature of predictions. Inasmuch as the USSR, which is a vast and ecologically diverse country, was used as the basis for

both modeling and prediction, it appears quite reasonable that similar computer models could also be built for other countries. This would require insignificant outlays and 2-3 years of time; absence of representative registration of influenza morbidity in many countries would be a complicating factor.

The average anticipation time of predictions for cities of the USSR is about 1 month. Thus for countries covering small territory, the national model would provide a significantly shorter prediction anticipation time. If it is found that this anticipation time is just a few days, the prediction would lose its meaning. However, each newly created national model could be immediately combined with those already in existence--this would require only an insignificant amount of information exchange. Through such action (naturally coordinated by the World Health Organization) we can gradually produce an international system of influenza epidemic prediction with an average prediction anticipation time of probably a few months. The participating countries could use the predictions in the interest of not only public health but also the economy.

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ANTIGENIC RELATIONSHIPS OF EPIDEMIC H1N1 VIRUS STRAINS ISOLATED IN NOVEMBER-DECEMBER 1977 IN THE USSR TO INFLUENZA VIRUSES OF HUMAN AND ANIMAL ORIGIN

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[Article by D. K. L'vov, V. M. Zhdanov, A. A. Sazonov, N. A. Braude, V. I. Reznik, T. V. Pysina, A. M. Osherovich, A. A. Berzin, I. A. Myasnikova, R. Ya. Podchernyayeva, V. P. Andreyev, S. M. Klimenko, M. A. Yakhno, Ye. A. Vladimirtseva, and L. V. Agafonova, Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow]

[Text] The antigenic relationships of four influenza virus strains (Moscow, Khabarovsk), which caused an epidemic outbreak in the USSR in November-December 1977, to influenza viruses of human and animal origin were studied using the hemagglutination inhibition test (HIT), and the neuraminidase activity inhibition test (NAIT). In the HIT, the antigens of the strains reacted with antisera to A/FM/1/47 (H1N1) to the homologous titer, with antiserum to the strain A/whale /Pacific Ocean/19/76 to one-eighth titer, with antiserum to A/Bel/42(HON1) to one-sixteenth titer, and with antiserum to A/PR/8/34 (HON1) from absence of specific interaction to one thirty-second titer. It was shown with NAIT that epidemic strains have type 1 neuraminidase, closer to the neuraminidase of the virus A/New Jersey/76(HswlN1) than to the virus A/FM/1/47. The pronounced relationship, in relation to hemagglutinin, of a type A1 virus, appearing in the human population for the first time, with a virus isolated from whales 1.5 years prior to this event, indicates the possibility for importation of a virus into the human population from natural ecosystems.

On 11 November 1977 a group from the Nakhodka Marine Fisheries School went to sea, and on 13 November 152 persons of this group returned ill from the cruise. On 14-20 November local influenza outbreaks were recorded among students in Khabarovsk and Vladivostok. According to reports from

T. V. Pysina (25 November, Vladivostok) and V. I. Reznik (28 November, Khabarovsk) the isolated strains were typed partially as A2 and partially as A1. This was the first information in the USSR concerning return of A1 virus to the human population after a 20-year interval. The strains were received by the ecology lab of the Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences on 8 December 1977. By this time, judging from the initial data similar strains were also isolated in Moscow. The four strains were studied with the hemagglutination inhibition test (HIT) and the neuraminidase activity inhibition test (NAIT) using a set of viruses of human and animal origin (8-13 December), including a virus we had isolated in 1976 from rorquals caught in winter 1975-1976 in the South Pacific. This article presents the results of our identification of epidemic strains and the influenza virus isolated from whales.

Materials and Methods

We studied the strains A/Moscow/0778/77, A/Moscow/0782/77, and A/Moscow /0897/77 isolated on 21-24 November 1977 from young patients in Moscow (A. M. Osherovich, City Public Health Station), and the strain Khabarovsk /03456/77 isolated on 24 November in Khabarovsk (V. I. Reznik, Oblast' Public Health Station) from a sick child. All strains were isolated in the first passage in chick embryos. Prior to analysis in the laboratories from which the material had been obtained, the strains had undergone one to two passages. The viruses were not passaged in our laboratory. Material from rorquals (family Baleanopteridae) was collected in winter 1975-1976 by a commercial expedition of the "Sovetskaya Rossiya" Whaling Flotilla (A. A. Berzin, Pacific Ocean Institute of Fisheries and Oceanography, Vladivostok). Seventy-two lung samples and 65 liver samples were taken from 72 whales and held until the time of virological analysis (4 months) at 20°C in flasks containing 50 percent glycerin, pH 7.2 phosphate buffer, and antibiotics (penicillin and streptomycin, 10,000 and 1,500 units/ml respectively). The viruses were isolated in 1977 in chick embryos. In all, we isolated 14 strains from 13 animals--13 out of lung samples and one out of a liver sample. The viruses were isolated in the second passage.

Antiserum to strain A/whale/Pacific Ocean/19/76 was prepared by threefold immunization of white rats. All 14 isolated strains were titrated with this serum to the homologous titer in the HIT (1:2560) and the NAIT (1:30). Thus the indicated prototype strain was used in the subsequent work.

A set of reference antisera to the following viruses was used in the HIT to identify the viruses: HON1-A/PR8/34, A/Bel/42*, R-5a (HONav1), N1N1-A/FM /1/47*, N2N2-A/Sing/1/57, H3N2-A/HK/1/68*, A/PCh/1/73, A/Tokyo/1/75, A/Victoria/75*, Hsw1N1-A/sw/Iowa/13/30*, A/sw/Wisc/67, A/NJ/76, Heq1Neq1-Eq/Prague/56*, Heq2Neq2-A/Eq/Miami/63*, Hav1N1-A/chick/FPV/Rostock/34, A/turk/Engl/63*, Hav4Hav1-A/Duck/Chech/56, Hav5Nav2-A/tern/SA/61*,Hav6Heg2-A /turk/Canada/63, A/Shearwater/Australia/72*, Hav7Neq2-A/Duck/Ukraine/63, Hav7Nav2*, Hav8Nav4-A/turk/Ontario/67*, Hav9Nav2-R136*.

*These sera were used in the NAIT.

Some of the antisera were obtained from doctors Webster and Kilbourne, to whom the authors express their gratefulness.

The HIT was performed according to the WHO procedure. The sera were treated with RDE. The NAIT was performed in accordance with a described method (1). Purified and concentrated virus A/whale/Pacific Ocean/19/76 was used for electron microscopy. The preparations were applied to a carbon Formvar base and contrasted with 1 percent uranyl acetate or methanol.

The methods used to study the biological properties of the viruses (eluting activity, capability for reproduction at different temperatures, plaque formation, and hemagglutinin heat stability) were published earlier (2).

Results

In our identification of the strain A/whale/Pacific Ocean/19/76 with HIT, we revealed antigenic relationships only with antisera to viruses of the antigenic complex HO-H1-Hsw1-Hav5, in which case the titer for antiserum to "late" virus HO(Bel/42) attains one-sixteenth of the homologous titer, while that for antiserum to "early" virus HO(PR/8/34) and H1 attains one thirty-second of the homologous titer (Table 1). Thus in relation to hemagglutinin the virus occupies as intermediate position between HO and H1, being closer to late HO.

The NAIT revealed that the neuraminidase of the virus A/whale/Pacific Ocean/19/76 is close or identical to type 2 avian neuraminidase (Nav2) (Table 2). Relationships were not revealed with other types of neuraminidase (N1, Neq1, Neq2, Nav1, Nav3, Nav4, Nav5).

Thus the antigenic formula of the virus A/whale/Pacific Ocean/19/76 (HO-H1, Nav2) had been known earlier for animal and human influenza viruses.

The electronogram revealed filiform and round virions with typical influenza virus thorns (see Figure 1). Comparative analysis of some biological properties revealed differences in reproduction between the virus isolated from whales and human influenza viruses at lower (28°C) and high (40°C) temperatures, and in the nature of plaques formed in chick embryo fibroblast cultures ("cloudy"). As with strain A/FM/1/47, the virus isolated from whales had thermoresistant hemagglutinin, in contrast to the thermolabile strain A/WSN/33(HON1). The virus was close to strain A/FM/1/47 with respect to elution rate with chick erythrocytes (Table 3).

In our identification of epidemic strains using the HIT, titers equal to the homologous titer were revealed with antiserum to virus A/FM/1/47 (H1N1). However, the virus works with antisera to viruses A/whale/Pacific Ocean/19/76 (HO-H1N1) and A/Bel/42 ("late" HON1) to 1/8 and 1/16-1/32 titer correspondingly (Table 4). Relationships with "early" virus

Table 1. Antigenic Relationship, in the HIT, of the Strain A/Whale/Pacific Ocean/19/76 with Viruses of the Complex H0-H1-Hsw1-Hav5*

(1) Тип гемагглютининов	(2) Референс-сыворотка	(3) Титры (обратные значения) антигемагглютининов с вирусом А/кит/Тихий океан/19/76	(4) Гомологичный титр
0	A/PR8/34	20	640
	A/Bel/42	80	1280
1	A/FM/1/47	20	640
sw1	A/Sw/Aowa/30	10	1280
av5	A/tern/SA/61	<10	80
(5)	А/кит/Тихий океан/19/76	2560	2560

*Negative results were obtained with reference antisera to viruses containing hemagglutinin types H2, H3, Heq1, Heq2, Hav1, Hav2, Hav3, Hav4, Hav5, Hav6, Hav7, Hav8, and Hav9.

Key:
1. Type of hemagglutinin
2. Reference antiserum
3. Titers (reciprocals) of anti-hemagglutinins to virus A/whale/Pacific Ocean/19/76
4. Homologous titer
5. A/whale/Pacific Ocean/19/76

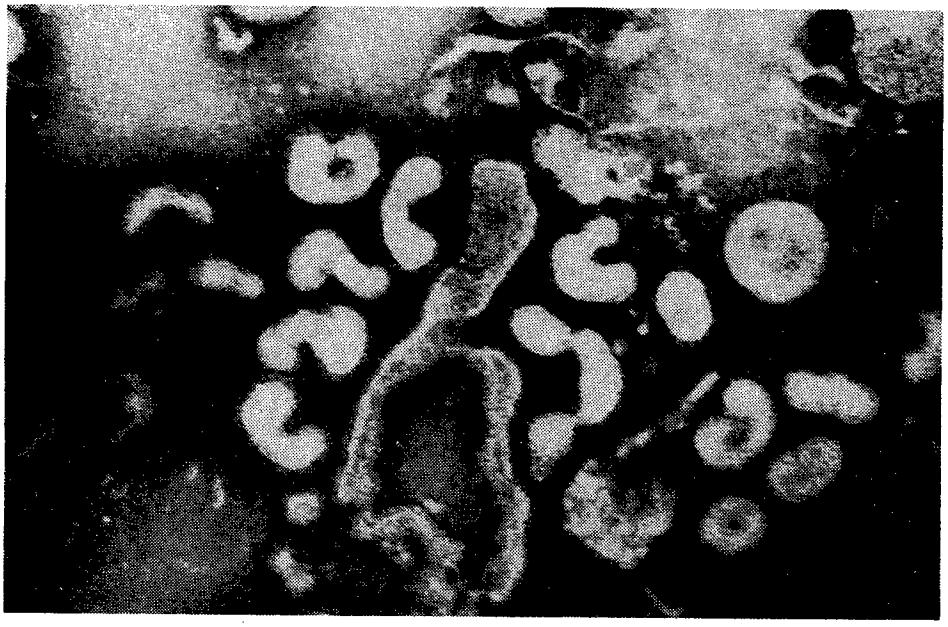
Table 2. Antigenic Relationships, in the NAIT, of the Strain A/Whale/Pacific Ocean/19/76 with Viruses with Type 2 Avian Neuraminidase*

(1) Тип нейраминидазы	(2) Референс-сыворотка	(3) Титр (обратные значения) антинейраминидазных антител с вирусом А/кит/Тихий океан/19/76	(4) Гомологичный титр
	A/tern/SA/61	30	30
Nav2	A/tern/Turkm/73	20	30
	R-3a (H0Nav2)	30	90
	R-136(Hav9Nav2)	30	60
(5)	А/кит/Тихий океан/19/76	30	30

*Negative results were obtained with reference antisera to viruses containing neuraminidase types N1, N2, Neq1, Neq2, Nav1, Nav3, Nav4, Nav5, and Nav6.

Key:
1. Type of neuraminidase
2. Reference antiserum
3. Titer (reciprocals) of anti-neuraminidase antibodies to virus A/whale/Pacific Ocean /19/76
4. Homologous titer
5. A/whale/Pacific Ocean/19/76

Figure 1. Virions of Virus A/Whale/Pacific Ocean/19/76



H0 (A-PR/8/34) were not revealed. Totally negative results were obtained with viruses containing all other known types of human and animal influenza virus hemagglutinins.

The NAIT revealed that the neuraminidase of epidemic strains is type N1 neuraminidase, closer to the neuraminidase of virus A/New Jersey/76 (HswlN1) than to that of virus A/FM/1/47 (H1N1)--that is, it is closer to the genealogically (but not chronologically) earlier viruses of the A0-A1 complex (Table 5).

Thus the epidemic strains have antigenic formula H1N1, in which case the hemagglutinin corresponds to the earlier variant H1, though it has a significant relationship to the late variant H0 and influenza virus isolated from whales, while the neuraminidase is more similar to the genealogical ancestors of the virus A1.

The proteins of the epidemic strain A/Moscow/0897/77 and the virus A/whale /Pacific Ocean/19/76 were analyzed in comparison with human influenza virus strains A/PR/8/34, A/FM/1/47, and A/WSN/33 using electrophoresis in 10 percent poylacrilamide gel.

Figure 2 shows an electrophoregram of proteins from the viruses A/WSN/33, A/PR/8/34, A/Moscow/0897/77, A/whale/Pacific Ocean/19/76, and A/FM/1/47. As we can see from Figure 2, the epidemic strain A/Moscow/0897/77 has a protein composition similar to that of strain A/FM/1/47. The virus

Table 3. Some Biological Properties of Influenza Virus A/Whale/Pacific Ocean/19/76 and Some Standard Viruses

(1) Вирус	(2) Титр гемагглютинина	(3) Бляшкообразование	rct, lg_{50} 28° C	37° C	40° C	(4) T_{56} гемагглютинин, мин	(5) E (100%), ч	(6) Репродукция в клетках
(7) А/кит/Тихий океан/19/76 (H0-H1Nav2)	1:640	+ (8) (мутные)	8,0	8,5	8,0	60	2	+
A/WSN/33 (H0N1)	1:640	+ (9) (прозрачные)	5,2	7,4	6,5	10	4	+
A/FM/1/47 (H1N1)	1:320	+ (9) (прозрачные)	6,5	7,0	6,5	120	1	+

Note: rct--capability for reproducing in chick embryos at different temperatures; T_{56}--thermoresistance of hemagglutinin to heating at 56°C for 30 minutes; E--elution with chick erythrocytes.

Key:
1. Virus
2. Hemagglutinin titer
3. Plaque formation
4. T_{56}, hemagglutinin, min.
5. Hours
6. Reproduction in cells
7. A/whale/Pacific Ocean/19/76
8. Cloudy
9. Translucent

A/whale/Pacific Ocean/19/76 is similar to the virus A/PR/8/34 in relation to the composition of all proteins except one, which is apparently neuraminidase. The protein of the virus A/whale/Pacific Ocean /19/76 is similar to neuraminidase, exhibits lower mobility in gel (that is, it has a higher molecular weight) as compared to the corresponding protein of human influenza viruses A/PR/8/34, A/FM/1/47, A/Moscow/0897/77, and it is apparently similar to neuraminidase of the virus A/WSN.

As we can see from Figure 2, both hemagglutinin subunits (HA_1 and HA_2) of strains A/Moscow/0897/77 and A/FM/1/47 have lower mobility in gel (that is, a higher molecular weight) as compared to the corresponding proteins of viruses A/PR/8/34 and A/whale/Pacific Ocean/19/76.

Discussion

In 1974-1976 we isolated viruses from wild animals in the Pacific Ocean basin antigenically similar to all known epidemic influenza viruses--the Hong Kong complex (H3N2) from terns (3), from the pintail duck (T. V. Pysina), and the virus (H2Nav2) from the pintail duck, similar in relation to hemagglutinin with the Asiatic virus H2N2 (4). The virus H0-H1Nav2 isolated from whales supplements this list.

Table 4. Identification* of Epidemic Strains with HIT (November 1977)

(1) Тип гемагглютинации	(2) Референс-сыворотка	(3) Титр (обратные значения) антигемагглютининов с антигенами эпидемических штаммов			(5) Гомологичный титр
		(4) А/Москва/0778/77	А/Москва/0782/77	А/Москва/0897/77	
H0	A/PR/8/34	<10	<10	20	640
	R-3a(H0Nav2)	<10	<10	<10	2560
	R-5a(H0Nav1)	<10	<10	20	1280
	A/Bel/42	80	40	80	1280
(6)	А/кит/Тихий океан/19/76 (H0—H1N1)	320	320	320	2560
H1	A/FM/1/47	640	640	640	640
H2	A/Sing/57	<10	<10	<10	1280
H3	A/HK/68	<10	<10	<10	5120
	A/PCh/73	<10	<10	<10	1280
	A/Tokyo/75	<10	<10	<10	160
	A/Vict/75	<10	<10	<10	160

*Negative results were obtained with reference antisera to viruses containing hemagglutinin types Hswl, Heql, Heq2, Havl Hav2, Hav3, Hav4, Hav5, Hav6, Hav7, Hav8, and Hav9.

Key:

1. Hemagglutinin type
2. Reference antiserum
3. Titer (reciprocals) of antihemagglutinins with antigens from epidemic strains
4. Moscow
5. Homologous titer
6. A/whale/Pacific Ocean/19/76

Figure 2. Electrophoregram of Influenza Virus Proteins: Ten percent polyacrilamide gel with SDS. Human influenza viruses: A/WSN (*1*, *10*), A/PR/8/34 (*2*, *6*), A/Moscow/0897/77 (*3*, *7*). A/FM/1/47 (*5*, *9*); influenza virus isolated from whales-- A/whale/Pacific Ocean/19/76 (*4*, *8*). Arrows indicated possible location of neuraminidase

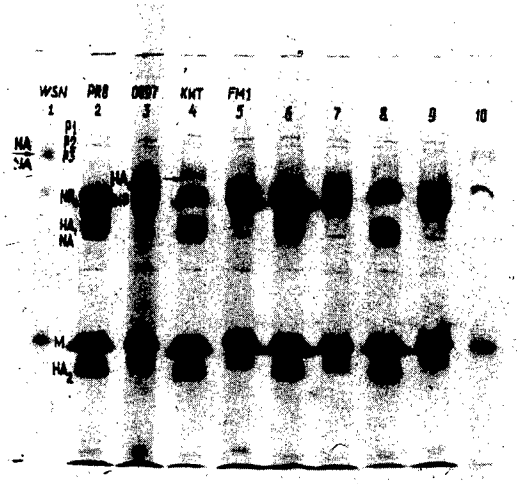


Table 5. Identification of Epidemic Strains (November 1977) Using Reference Antisera in the NAIT

(1) Тип нейраминидазы	(2) Референс-сыворотка	(3) Обратные значения титров антинейраминидазных антител с эпидемическими штаммами				(6) Гомологичный титр
		(4) А/Москва/0778/77	(4) А/Москва/0783/77	(4) А/Москва/034561/77	(5) А/Хабаровск/034561/77	
N1	A/FM/1/47	60	30	20	30	90
	A/NJ/76	60	20	60	30	30
	X-53(Hsw1N1)	20	30	60	30	120
	A/FPV/Rostok/34	20	30	60	<10	—
	R-6a(Hav7N1)	20	20	30	10	30
	R-2069(H3N1)	30	20	20	20	60
N2	A/Sing/57	<10	<10	<10	—	60
	A/HK/68	<10	<10	<10	> —	60
	A/PCh/73	<10	<10	<10	—	100
	A/Tokyo/75	<10	<10	<10	—	40
	A/Vict/75	<10	<10	<10	—	20
Neq1	A/eq/Pr/56	<10	<10	<10	—	60
Neq2	A/eq/Mi/63	<10	<10	<10	—	50
Nav1	A/duck/Eng/56	<10	<10	<10	—	30
Nav2	A/tern/SA/61	<10	<10	<10	<10	30
	A/Anas acuta/Primorje/76	<10	<10	<10	<10	60
	R-136(Hav9Nav2)	<10	<10	<10	<10	60
	R-3a(H0Nav2)	<10	<10	<10	<10	90
	A/turn/Eng/63	<10	<10	<10	—	40
	A/turn/Ont/67	<10	<10	<10	—	60
	A/Shearw/Aust/72	<10	<10	<10	<10	20
	A/duck/Memplis/75	<10	<10	<10	<10	90

Key:

1. Neuraminidase type
2. Reference antiserum
3. Reciprocals of the titers of antineuraminidase antibodies with epidemic strains
4. Moscow
5. Khabarovsk
6. Homologous titer

Thus, viruses which had disappeared from the human population 10-20 years ago continue to circulate in natural ecosystems in the region from which new epidemic influenza viruses originate. It is entirely probably that as a result of complex ecological processes, including recombination, some proportion of the strains out of the tremendous diversity of the heterogenic population of influenza viruses could readapt itself to the human body. Could this not have been the case with the new epidemic virus H1N1, which appeared among people once again after a 20 year interval and 1.5 years after isolation of a whale virus similar in antigenic respects? The virus could be transmitted to whales by birds, the virus-containing feces of which drop to the water in huge quantities at marine colonial nesting grounds. An intermediate role on the part of plankton is not excluded either (5).

The duration of the human circulation cycle of the modern variant of the virus H1N1 will hardly exceed 2-3 years, being limited through the population's immunity to persons older than 20 years. After this period a new variant should replace it.

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COMPARATIVE STUDY OF B-LYMPHOCYTE ACTIVITY IN THE SPLEEN OF MICE INFECTED BY VIRUSES OF THE TICK-BORNE ENCEPHALITIS COMPLEX

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 207-211

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[Text] A description is given of the differentiated reaction of B-cells of mouse spleen to equivalent doses (100 LD_{50}) of virulent and attenuated strains of tick-borne encephalitis complex viruses, investigated by the method of passive local hemolysis. Immunization of the mice was accompanied by an active reaction of spleen B-cells and formation of specific resistance without a noticeable increase in the level of antihemagglutinins circulating in the blood.

It has been established in recent years that in addition to B-lymphocytes, which are responsible for development of humoral immunity, T-lymphocytes, which are responsible for development of cell immunity, play an important role in development of resistance to arbovirus infections (1,2).

The mechanisms of immunity to arbovirus infections, particularly those elicited by various attenuated strains, are still insufficiently studied today. This pertains first of all to the factors of cell immunity and their relationship to overall resistance and the level of humoral antibodies.

Research on the pathogenesis of togavirus infections, including those elicited by viruses of the tick-borne encephalitis (TE) complex, revealed pronounced tropism of agents in relation to tissues of the reticuloendothelial system, including the spleen, in which the virus is not only localized but also multiplied (3-5).

The objective of our research was to study the effect of certain viruses of the TE complex having different degrees of virulence on the antibody-forming activity of spleen B-lymphocytes, revealed by a modified passive local hemolysis method on the basis of the quantity of antibody-forming or so-called plaque-forming cells (PFC) in a suspension of mouse splenocytes. In addition we revealed the relationship of the level of PFC to the overall resistance of the body and the level of humoral antihemagglutinins.

Materials and Methods

Viruses and animals: We used three strains of TE complex viruses exhibiting different degrees of neurovirulence: The highly virulent Pan strain of TE virus, naturally weakened by Tp-21 strain Langat virus, and a highly attenuated variant of the latter, Tp-21-237 (6). In addition we used Absettarov strain TE virus to test resistance.

The research was conducted on white mongrel mice in two weight categories--6-7 and 18-20 gm. These weight categories were chosen because young mice weighing 6-7 gm are highly sensitive to TE complex viruses and are used most frequently in various virological studies. Sexually mature mice weighing 18-20 gm are used most often as a model for studying various problems in immunology.

Methods: Strains Pan, Tp-21, and Tp-21-237 were injected subcutaneously into mice of the two weight categories in equal doses--100 LD_{50}/0.25 ml. When two virus injections were given, the interval between injections was 10 days.

Different doses of Absettarov strain were injected intraperitoneally to test the resistance of immunized animals.

Humoral immunoglobulins were determined by the hemagglutination inhibition test, using the commonly accepted procedure, with antigens corresponding to those used for immunization purposes. Macroglobulins were disintegrated with 2-mercaptoethanol to permit differentiation of macro- and microglobulins (7).

Blood was drained completely from the animals and the spleens were removed on the 3rd, 5th, 7th, 10th, 14th, and 21st days after inoculation of the viruses in order to determine the quantity of PFC (Figure 1), which would provide an indication of the activity of intracellular synthesis of antibodies. Splenocytes were obtained by a "scraping" method followed by two-time rinsing with medium 199 in Henk's solution. To reveal PFC, we used (Genneygeym's) local hemolysis technique, modified in relation to certain details of the method (8-11), as being the most sensitive and efficient technique. Sheeperythrocytes initially treated with the antigen used for immunization were employed as the test antigen (12-14). A 10

percent virus suspension of mouse brain at a 1:5 dilution was used as the antigen. After a period of adsorption of the antigen on erythrocytes for 1 hour at 37°C and three rinsings with physiological solution, the erythrocytes were stored at 4°C; a 1:2 dilution was used in the experiments.

The splenocyte reaction was expressed as a percent ratio of PFC to the quantity of nucleated cells in a preparation prepared in parallel. Both indices were determined in five fields of view of each preparation. To count nucleated cells, we stained them in a Giemsa stain solution having a final dilution of 1:3.

We examined animals separately and derived average indices ($\bar{x}$) and the standard deviation (S) to describe the natural variability of the particular index, and we also determined the significance of differences in relation to PFC indices of intact animals (differences were interpreted as significant when $P \leqslant 0.05$). Analysis of the reaction of splenocytes from intact mice showed that the spleens of such animals contained cells capable of spontaneous hemolysis, and that their number varies in individuals and in different age groups.

Results and Discussion

Using the modified passive local hemolysis technique, we established from the PFC indices that mouse spleen B-lymphocytes respond to the initial antigenic action of a 100 LD_{50} dose of TE complex viruses (Pan, Tp-21, Tp-21-237), injected subcutaneously, with a pronounced specific reaction. The dynamics and intensity of the reaction varied depending on the viruses and the age of the animal.

Thus in mice weighing 6-7 gm PFC appear and reach a high level (10-15 percent as compared to 3 percent in intact animals) earlier than in animals weighing 18-20 gm.

A more highly pronounced reaction of B-lymphocytes was noted in mice weighing 18-20 gm in response to injection of the same viral doses (100 LD_{50}), the PFC indices being 19-20 percent and higher; the reaction developed somewhat later, beginning with the 7th day, and remained at a rather high level until the 14th day after infection (see table).

The highest antigenic activity was noted in this group of animals for the attenuated variant of Langat virus, Tp-21-237.

The earliest pronounced reaction (within the first 3 days) of B-lymphocytes in response to injection of Tp-21 strain Langat virus was among animals weighing 6-7 gm. When animals were infected with this strain, the highest PFC indices and a significant difference with respect to the PFC level of the

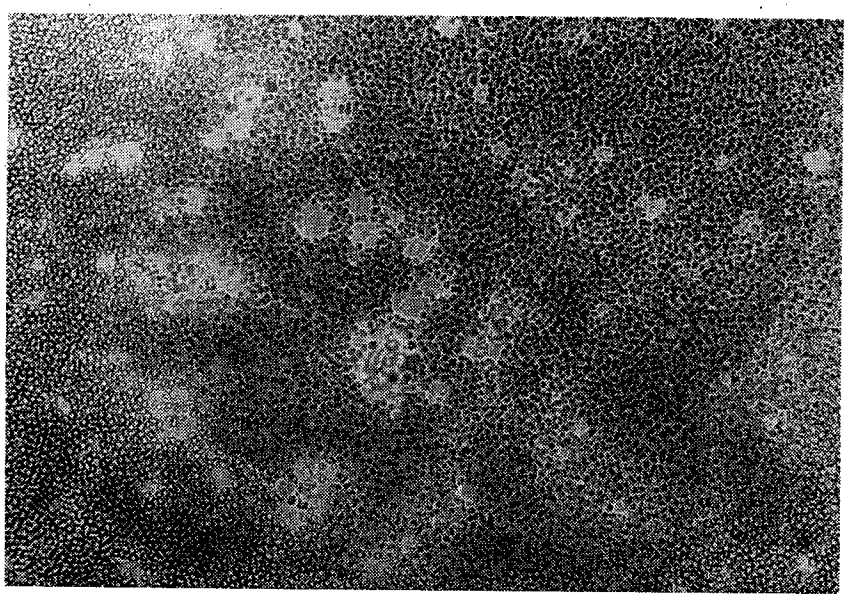

Figure 1. Zones of Local Hemolysis: Plaques on the 10th day following injection of strain Pan; magnification 200×

intact animals were noted on the 1st, 2nd, 8th, and 15th days; these phenomena were noted on the 3rd, 6th, 8th, and 15th days in animals infected with strain Tp-21-237, and on the 8th, 10th, and 13th days in animals infected with Pan strain. The possibility exists that the most virulent strain somewhat inhibits the activity of immunocompetent spleen cells in the first days following its injection into the animal's body, while strain Tp-21 has a stimulatory effect on these cells.

We also studied the reaction of spleen B-cells to reimmunization of the animals with a 100 LD_{50} dose of homologous virus. We found that secondary injection of strain Tp-21-237 into mice weighing 6-7 gm elicited an increase in the level of PFC, the number of which following reimmunization exceeded the PFC level at the same time following the initial antigen exposure (Figure 2). Thus immunocompetent cells carry a trace of the antigenic stimulus, inasmuch as they are capable of a revaccinational form of response. Apparently a 100 LD_{50} dose of attenuated strain Tp-21-237 can also elicit formation of an immunological memory in the cells at the first immunization.

The reaction of splenocytes to secondary antigenic stimulation turns out to be somewhat different for animals weighing 18-20 gm (Figure 3). The highest PFC level was observed for animals in this weight category at the moment of reimmunization (10th day). A secondary noticeable rise in their level was noted in this connection.

Table 1. Comparative Characteristics of the Reaction of Mouse Spleen B-Lymphocytes to the Antigenic Action of TE Complex Viruses.

(1) День после введения вируса	(2) Штамм Пан				(3) Штамм Тр-21				(4) Штамм Тр-21-237			
	(5) весовая категория мышей											
	6–7 г (6)		18–20 г		6–7 г		18–20 г		6–7 г		18–20 г	
	$\bar{x} \pm S$	P	$\bar{x} \pm S$	P	$\bar{x} \pm S$	P	$\bar{x} \pm S$	P	$\bar{x} \pm S$	P	$\bar{x} \pm S$	P
1-й	7,7±4,0	<0,05	—		10,0±2,0	<0,05	—		4,5±0,7	>0,005	—	
2-й	6,1±3,6	>0,05	—		10,3±3,1	<0,05	—		2,7±2,3	>0,05	—	
3-й	6,7±4,1	>0,05	0,3±0,8	>0,05	10,0±7,4	<0,05	0,4±0,3	>0,05	11,4±7,3	<0,05	1,2±1,2	>0,05
4-й	—		0				0,7±1,2	>0,05	—		0,3±0,9	>0,05
5-й	—		1,8±2,7	>0,05	—		0,5±0,7	>0,05	—		3,2±2,7	>0,05
6-й	3,4±3,1	>0,05	0		8,1±9,2	<0,05	0,7±1,1	>0,05	9,8±11,1	>0,05	0,3±0,3	>0,05
7-й	3,9±4,3	>0,05	9,7±7,7	>0,05	2,8±1,9	>0,05	19,8±42,9	>0,05	7,7±4,4	>0,05	3,4±1,8	>0,05
8-й	8,1±2,6	<0,05	—		8,25±7,5	<0,05	—		8,7±9,5	>0,05	—	
9-й	—		—		—		—		—		—	
10-й	15,3±5,9	<0,05	19,4±8,6	<0,05	6,6±6,0	>0,05	13,3±10,7	>0,05	5,6±6,0	>0,05	21,5±7,1	<0,05
11-й	—		14,4±12,1	<0,05	—		10,9±0,3	0,05	—		80,7±7,1	<0,05
12-й	—		10,4±11,1	>0,05	—		19,8±20,6	0,05	—		13,4±10,6	<0,05
13-й	15,2±2,4	<0,05	10,6±10,5	>0,05	12,2±9,52	<0,05	9,6±6,3	0,05	7,9±1,6	<0,05	11,6±8,1	<0,05
14-й	3,1±2,4	>0,05	18,1±13,3	<0,05	4,4±1,8	>0,05	7,0±1,8	>0,05	6,6±6,8	>0,05	9,0±10,7	>0,05

Key:

1. Day after virus injection
2. Pan strain
3. Tp-21 strain
4. Tp-21-237 strain
5. Mouse weight category
6. gm

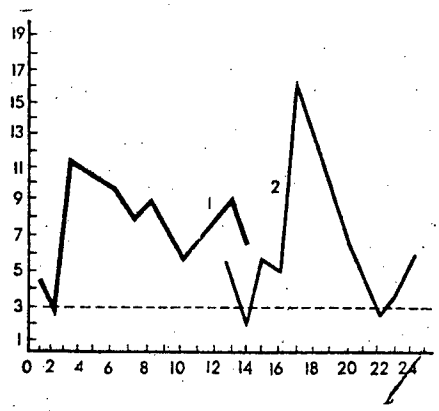

Figure 2. Dynamics of the intensity of the PFC reaction to initial (*1*) and secondary (*2*) injection of strain Tp-21-237 into mice weighing 6-7 gm: Here and in Figure 3, the broken line indicates the PFC level of unimmunized mice; ordinate--percent ratio of PFC to the quantity of nucleated cells; abscissa--days after virus injection.

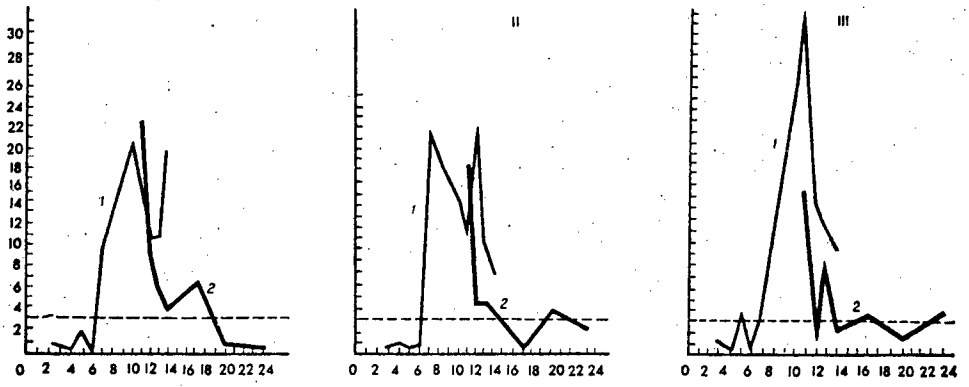

Figure 3. Dynamics of the intensity of the PFC reaction to initial (*1*) and secondary (*2*) injection of strains Pan (*I*), Tp-21 (*II*), and Tp-21-237 (*III*) into mice weighing 18-20 gm.

We were also interested in the relationship of the quantity of PFC or the intensity of the inductive intracellular phase of antibody formation to the intensity of the productive phase or the level of humoral antibodies, particularly antihemagglutinins in immunized animals. With this purpose we performed hemagglutination inhibition tests at the appropriate time on animals of the two weight categories, infected by strains Pan, Tp-21, and Tp-21-237, in order to reveal presence of macro- and micorimmunoglobulins (IgM and IgG). We noted that at the observed PFC levels, antihemagglutinins

to the indicated viruses were revealed irregularly, in titers not exceeding 1:10.

It is possible that 100 LD_{50} doses of the viruses are insufficient to induce a pronounced productive phase of antibody formation. It is also highly probable that the sensitivity of the hemagglutination inhibition test is too low to reveal minimum quantities of antihemagglutinins in blood.

Of interest is the fact that 2 days after one-time subcutaneous immunization with a 100 LD_{50} dose of TE complex viruses (Pan, Tp-21, Tp-21-237), despite absence or irregular detection of humoral antihemagglutinins, mice in both weight categories (6-7 and 18-20 gm) turned out to be resistant to intraperitoneal injection of the virulent Absettarov test strain TE virus.

The resistance index of mice weighing 6-7 gm immunized with strain Tp-21-237 once was 3.6 lg LD_{50}, reaching 6.0 lg LD_{50} 2 weeks after two-time immunization. Experiments aimed at revealing infectious virus and interferon in the spleen of immunized mice produced negative results. Obviously a 100 LD_{50} virus dose is not enough for active multiplication of the virus in the animal's body, for induction of interferon, and for initiation of the productive phase of antibody formation. At the same time the 100 LD_{50} dose of these viruses is capable of causing activation of mouse spleen B-lymphocytes and intracellular synthesis of antibodies in a significant proportion of these cells, as well as resistance to subsequent intraperitoneal injection of virulent TE virus. Resistance apparently developed with the help of T-lymphocytes, which are responsible for development of cell immunity; the protective role of such resistance in relation to a number of flavivirus infections, including TE, has been demonstrated by a number of authors (1-3).

Our research showed that small doses (100 LD_{50}) of TE complex viruses can have a certain antigenic effect on mouse spleen B-cells when injected subcutaneously. Certain differences were noted in the dynamics and intensity of stimulation of B-lymphocytes by virulent and attenuated virus strains. Absence of noticeable growth in the level of antihemagglutinins in the blood of immunized animals in the presence of the development of resistance with indices up to 5.0 and higher attests to the important role played by cell immunity in relation to TE, and to the need for deeper research on general resistance, antibody formation, B-cell reaction, and T-system immunity.

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OXIDATIVE PHOSPHORYLATION AND CHEMOLUMINESCENCE IN EXPERIMENTAL INFLUENZA INFECTIONS

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 157-160

[Article by N. P. Zalyubovskaya, R. I. Kiselev, and L. N. Turchaninova, Khar'kov Scientific Research Institute of Microbiology, Vaccines, and Sera imeni I. I. Mechnikov]

[Text] The state of metabolic processes in the cell goes a long way in defining the activity with which a virus infection develops. In research on the pathogenesis of virus infections, the nature of energy metabolism, which is closely associated with the metabolic function of mitochondria, is quite interesting. We can judge the specific features of energy metabolism in response to development of virus infection on the basis of a study of the functional activity of liver mitochondria existing in different metabolic states. Biochemoluminescence is a normal property of cells and tissues, and the energy for it is provided by free-radical oxidation of lipids, mainly phospholipids, which can participate in electron transport in the respiratory chain (1,2). The opinion exists that luminescence of a suspension of mitochondria is associated with formation of fatty acid peroxides, which depends on oxidative phosphorylation (3). Of interest are facts such as change in the state of oxidative phosphorylation in the liver of animals poisoned by staphylococcus toxin (4), and higher intensity of chemoluminescence of blood serum from tuberculosis patients (5) as well as sap in leaves afflicted by a plant polyvirus infection (6). These facts demonstrate that when infectious processes develop within the organism, changes occur in the state of energy metabolism. However, the cases of disturbances in bioenergetics, encountered in the literature and assessed on the basis of biophysical criteria, are fragmentary and unassociated.

The goal of the present work was to study the relationship between oxidation and phosphorylation in mitochondria of chick embryo liver tissue, and the intensity of chemoluminescence of allantoic fluid in the dynamics of development of experimental influenza infection.

Materials and Methods

Victoria strain type A2 influenza virus passaged in the allantoic cavity of chick embryos was employed. The initial hemagglutination inhibition titer of the virus was from 1:320 to 1:640.

Infection: Nine day old chick embryos were infected with virus-containing allantoic fluid at a volume of 0.1 ml which corresponded to 2.0 lg (EID_{50}).

Mitichondria were obtained from homogenized liver tissue by differential centrifugation (7) in a medium containing 0.25 M sucrose and $1 \cdot 10^{-3}$ M EDTA (pH 7.4). After EDTA was rinsed from the mitochondria the latter were suspended in 0.25 M sucrose and stored refrigerated.

The oxygen consumption rate was recorded polarographically using a rotating platinum electrode. The incubation medium contained 58.3 mM tris-Hcl, 80 mM KCl, 0.7 mM EDTA, 1.6 mM $MgCl_2$, 42 mM sucrose, and 20 mM KH_2PO_4. Sodium succinate (20 mM) served as the oxidation substrate, and ADP served as the phosphate acceptor. Protein was determined in accordance with Lowry's method. Respiration rate was expressed in nanoatoms of oxygen per minute per milligram of protein. Phosphorylation intensity was computed as the rate of ADP consumption per minute per milligram of protein.

Chemoluminescence was recorded with a quantometric device consisting of a thermostatic measuring cuvette and an FEU-42 low-noise photoelectronic multiplier, an LPU-01 amplifier, and an EPP-09 recorder. Centrifuged allantoic fluid was measured at 37°C.

The significance of differences in the mean values of chemoluminescence of embryonic allantoic fluid was established by assessing confidencecoefficients. Student's and Fisher'stests were used to statistically treat the results (8). The results were considered to be significant at $P<0.05$.

Results

Oxidation and oxidative phosphorylation by liver mitochondria were studied on the 1st, 3d, 4th, and 5th days following infection of chick embryos with influenza virus. Uninfected chick embryos served as a control.

The developmental dynamics of influenza infection revealed a decline in the oxidative activity of liver mitochondria (see Table).

The data in the Table are averages for three parallel experiments, with 50 embryos infected in each experiment. When ADP was added, the respiration intensity of control mitochondria increased by 2.9 times--that is, exogenous ADP significantly accelerated electron transport along the respiratory chain, indicating that liver mitochondria have a high capability for phosphorylation associated with oxidation. This is also confirmed by the high value

Change in Functional State of Chick Embryo Liver Mitochondria in the Dynamics of Influenza Infection Development (M±m)

(1) День после заражения	(2) Количество O_2, натом/мин на 1 мг белка			(3) Дыхательные контроли		(4) АДФ/О	(5) Интенсивность фосфорилирования
	V_2	V_3	V_4	V_3/V_4	V_3/V_2		
(6) Контроль	3,08±0,20	8,88±0,61	4,44±0,12	2,0±0,06	2,85±0,03	1,97±0,02	17,23±1,10
1-й	2,45±0,23	6,85±0,59	3,63±0,10	1,88±0,05	2,79±0,04	1,93±0,03	13,23±0,97
3-й	1,72±0,27	4,01±0,57	2,13±0,15	1,88±0,06	2,33±0,05	1,58±0,04	6,31±0,54
4-й	1,66±0,25	3,24±0,62	1,90±0,19	1,71±0,04	1,96±0,03	1,58±0,02	5,10±0,82
5-й	1,68±0,21	3,38±0,58	1,97±0,17	1,72±0,06	2,01±0,05	1,60±0,03	5,40±0,76

Key:

1. Days after infection
2. Quantity of O_2, nanoatoms/min per mg protein
3. Respiratory controls
4. ADP/O
5. Phosphorylation intensity
6. Control

of the conjugation coefficient (V_3V_4) characterizing the capability mitochondria have for switching from an active phosphorylating state to an adjusted state. Introduction of 2,4-dinitrophenol (DNP) into the incubation medium of control mitochondria increased the respiration rate by 2.5 times as compared to that both on the background of succinate and after depletion of the ADP supply. The intensity of phosphorylation is maximum in control. By as early as 1 day after infection, the capability of mitochondria for oxidation of the substrate declined by 20.5 percent as compared to control. On the 4th day after infection the rate of oxygen consumption on the background of succinate declined by 46 percent, remaining at this level on the 5th day.

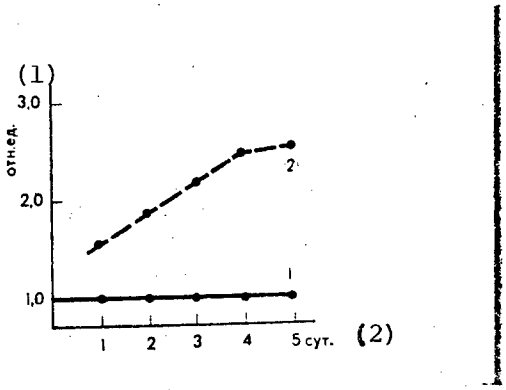
As influenza infection developed, the reaction of mitochondria to addition of ADP and DNP weakened, the intensity of oxidation in the active phosphorylating state (V_3) declined by 23-64 percent, and the oxygen consumption rate following depletion of ADP (V_4) decreased by 18-57 percent as compared to control. The maximum decline in the level of energy metabolism of the mitochondria was noted on the 4th day of development of the influenza virus. By this time the use of ADP by mitochondria in infected embryos decreased by 70 percent as compared to control.

As the influenza infection developed, the interdependence of the processes of oxidative phosphorylation was observed to undergo dramatic disturbance at all times; this was indicated by a decline in the values of the respitory controls. On the 4th - 5th days of development of influenza infection the values of the respiratory controls characterizing the capability of mitochondria for conjugation decreased by 1.2 times, while phosphorylation intensity decreased by 3.4 times. In the dynamics of chick embryo infection, the phosphorylation rate changed more than did the oxidation rate, resulting in a decline in the ADP/O coefficient.

The intensity of chemoluminescence of allantoic fluid depended on the time after influenza infection. The intensity of chemoluminescence of allantoic fluid was low but sufficiently stable for uninfected chick embryos (see figure). After the chick embryos were infected with influenza virus, the intensity of chemoluminescence of allantoic fluid increased over that of control. As the virus infection developed, the intensity of chemoluminescence increased, attaining a maximum on the 4th day of infection. We can assert with a probability close to 100 percent on the basis of the computed confidence coefficient that the differences in the average intensity of chemoluminescence of allantoic fluid from chick embryos infected with influenza virus are not random.

Discussion

The research established that the liver mitochondria of uninfected embryos have greater possibilities for self-regulation of energy processes. This



Chemoluminescence of allantoic fluid from chick embryos not infected (1) and infected by influenza virus (2)

Key:
1. Chemoluminescence intensity, relative units
2. Days

is evidenced by the high value of coefficient V_3/V_4 and the significant phosphorylation rate. The ATP macroergs formed in this case can be used in biosynthetic processes.

As influenza infection develops, the coefficients of the respiratory controls and phosphorylation intensity declined. These indices change most significantly on the 4th - 5th days after infection. This much time after infection, embryonic liver mitochondria are characterized by a sharp decrease in conjugation of oxidation and phosphorylation.

It becomes obvious from these data that influenza virus has an inhibitory effect on energy metabolism of liver mitochondria in chick embryos. It was manifested especially clearly on the 4th-5th days of infection, and it was expressed as a decline in conjugation of oxidation and oxidative phosphorylation. Obviously the decline in oxidation intensity stems from disturbance of electron transport in the same link of the respitory chain in which phosphorylation is disturbed. The decrease in the coefficients of respiratory controls could be a consequence of inhibition of electron transport along the respiratory chain during development of influenza infection; in this case the intensity of respiration in an active phosphorylating state using ADP declines more than does the intensity of respiration on the background of the substrate and following depletion of ADP, since the latter respiration rates are also regulated by the phosphate potential.

The noted increase in intensity of chemoluminescence of allantoic fluid in response to development of influenza infection may be associated with structural molecular changes in the lipoprotein complexes of cell membranes, and it probably is the result of exclusion of the lipid component. Data exist

indicating destruction of cell membranes during development of a virus infection (9). We found that by affecting the membrane lipoprotein complex, viruses promote a significant increase in the quantity of free fatty acids and an increase in the relative concentration of palmitic, linoleic, and oleic acids (10).

Inasmuch as lipids are the principal substrate in oxidative reactions accompanying chemoluminescence (1,2), we can hypothesize that destruction of membranes by influenza virus is accompanied by introduction of additional substrate and, as a result, by an increase in the intensity of chemoluminescence of allantoic fluid.

Thus basing ourselves on data in this article, we suggest that when it develops in chick embryos, influenza virus acts upon the cell's membrane structures. As a result of change in permeability and destruction of membrane structures during viral reproduction, electron transport in the respiratory chain becomes disturbed, leading to a decline in the intensity of oxidative phosphorylation; lipid components are also released from lipoprotein complexes, promoting intensification of chemoluminescence of allantoic fluid. Obviously, development of influenza virus is associated with suppression of the bioenergetic state in the tissues and organs of the infected organism.

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INVESTIGATION OF THE ANTIGENIC SPECIFICITY OF THE HEMAGGLUTININ OF TYPE A INFLUENZA VIRUSES BY QUANTITATIVE RADIOIMMUNOLOGICAL ANALYSIS

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 160-165

[Article by O. N. Berezina, V. V. Blokha, S. S. Yamnikova, M. A. Yakhno, and L. Ya. Zakstel'skaya, Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow]

[Text] Successful application of the method of isotopic dilution to determine very small antigen quantities in an antigen-antibody complex permitted development of the grounds for quantitative radioimmunological analysis (RIA). This method has advantages over other immunological methods owing to high sensitivity, specificity, precision, and productivity. The RIA method permits not only precise determination of extremely small antigen quantities, but also concurrent analysis of the degree of antigenic kinship of various biological materials.

The RIA method has not come to enjoy widespread application in oncovirology and in hepatitis research. As far as influenza is concerned, research in this direction is just beginning. Use of RIA in influenza virus research has been described in two publications (1,2). However, the antigen used in the two cases was not immunologically the same: It was either a virus labeled with ^{125}I or a hemagglutinin preparation highly contaminated by neuraminidase.

Because of the perpetual antigenic variability of influenza virus, which affects proteins on the outer surface of the virion--hemagglutinin and neuraminidase--and which results in epidemically active variants of type A influenza virus, individual antigenic determinants must be identified.

In this connection the goal of our research was to try to assess the possibilities for quantitative radioimmunological analysis in research on the content and immunological characteristics of hemagglutinin contained in epidemic strains of human group H3N2 type A influenza virus containing homologous hemagglutinin, in comparison with influenza virus A/PR8 containing antigenically distinctive hemagglutinin. We used highly purified hemagglutinin from influenza A/MRC-11 (H3N2) and monospecific antiserum as the standard test system for RIA.

Materials and Methods

Viruses: We used the following type A influenza: A/PR8 (H0N1), A/Port-Chalmers/1/73 (H3N2), and the recombinant A/MRC-11 (H3N2) (A/Port-Chalmers/ /1/73--A/PR8), which retained the antigenic properties of A/Port-Chalmers/ /1/73 and possessed the high reproductive capability of A/PR8. In our experiments we used both allantoic fluid containing these strains and purified concentrated preparations.

Hemagglutinin: Influenza virus A/MRC-11 was used to obtain hemagglutinin. The virus-containing allantoic fluid was clarified, and the virus was concentrated into a plaque by centrifugation with an SW25 rotor and a Bekman L5-50 centrifuge at 25,000 rpm for 1 hour. The precipitate was suspended in pH 7.2 0.1 M phosphate buffer containing 0.15 M NaCl, and further purification was performed by centrifugation in a linear 15-40 percent sucrose gradient with an SW27 rotor at 19,000 rpm for 4 hours. Fractions containing the virus were concentrated at 25,000 rpm for 1 hour. The precipitate was resuspended and used to obtain hemagglutinin by the technique suggested by Brand and Skehal (3). The purity of the obtained preparation was determined by electrophoresis in polyacrilamide gel (4). The hemagglutinin preparation contained 120 mg of protein per ml.

Antiserum: Antihemagglutinating serum was obtained by immunizing rats with purified hemagglutinin at a dose of 80 µg protein per injection. Immunization was repeated 1 month later. The obtained serum was tested in serological reactions and with the immunodiffusion reaction (5).

The obtained serum contained precipitating and antihemagglutinating antibodies at 1:5,120-1:10,240 titers, and it did not contain antineuraminidase antibodies.

Protein iodination: Hemagglutinin was labeled with ^{125}I *in vitro* using chloramine T as the oxidant in accordance with the method, somewhat modified, developed by Hunter (6) to label microquantities of proteins.

Iodination was performed with $Na^{125}I$ solution without a carrier, pH 8.0-11.0, with specific activity of 119 (mKi)/ml (9.9 (Ki)/mg I), supplied by the All-Union Izotop Association.

To obtain labeled preparations we mixed 50 µl 0.5 M sodium phosphate buffer (pH 7.5), 15 µl $Na^{125}I$ (119 mKi), and 15 µl (6 µg) hemagglutinin. We added 25 µl (100 µg) chloramine T to the solution and incubated it for 30 seconds at room temperature while shaking vigorously. Immediately after incubation, the reaction was halted by addition of 100 µl (250 µg) sodium metabisulfite and 100 µl (200 µg) KI. Labeled hemagglutinin was separated from unreacted iodine and from decomposition products formed during iodination, using gel filtration in a column containing Sephadex G-100 buffered with pH 7.5 0.05 M phosphate buffer. The fractions were collected in 1 ml increments under the control of a gamma-counter. Fractions containing the first radioactivity peak were combined, and bovine serum albumin (BSA) was added to them to a 0.1 percent

concentration. All vessels and the column were initially rinsed with a 1 percent BSA solution to prevent loss of protein owing to adsorption on glass.

The degree of dilution of free and bound ^{125}I was checked on the basis of precipitation of the material in 10 percent trichloroacetic acid: The preparation was considered to be usable if it contained not less than 90 percent acid-soluble radioactivity.

The quality of the obtained ^{125}I-hemagglutinin was tested for radiochemical purity by eletrophoresis in polyacrilamide gel, and for specificity of precipitation by antihemagglutinin serum. In our subsequent investigation we used only radiochemically pure preparations of ^{125}I-hemagglutinin for which nonspecific binding of the label was less than 10 percent. The specific activity of iodized preparations was $7 \cdot 10^6$ disintegrations per minute per microgram of protein.

Quantitative radioimmunological analysis: Buffer for the RIA was prepared with triple-distilled water and the following components: 0.01 M phosphate buffer (pH 7.5), 0.1 M sodium chloride, 0.001 M EDTA, 0.01 percent sodium azide, 0.01 percent BSA, and 0.01 percent Triton X-100.

Considering that in an RIA we are dealing with a soluble antigen-antibody complex, we separated free ^{125}I-hemagglutinin and ^{125}I-hemagglutinin bound to antibodies by indirect immunoprecipitation; antispecific antiserum to rat γ-globulin was used as a precipitating agent. The quantity of antispecific antiserum forming a precipitate was determined by preliminary titration with normal rat serum diluted to 1:100.

Before performing the RIA we determined the working dilution of immune serum. For this purpose we titrated a constant quantity of ^{125}I-hemagglutinin with serial dilutions of antisera, following this with separation of bound and free labels. In the RIA we used an antihemagglutinin serum dilution capable of binding 50 percent of the labeled hemagglutinin into an immune complex. In our case it was 1:15,000.

The RIA was performed according to the general procedure with ingredients added in the following sequence: 250 µl of the working dilution of antihemagglutinin serum in RIA buffer containing normal serum at a dilution of 1:100; 10 µl unlabeled hemagglutinin or the sample under analysis (competitor); 10 µl ^{125}I-hemagglutinin having an activity of 5,000-10,000 pulses/min. The mixture was incubated for 1 hour at 37°C and for 12 hours at 4°C. After 100 µl of antispecific serum was added, the mixture was once again incubated for 1 hour at 37°C, and not less than 4 hours at 4°C. Precipitate containing labeled hemagglutinin bound into an immune complex was precipitated by centrifugation at 2,500 rpm for 20 minutes. The supernatant was sucked or poured out, and bound radioactivity in the precipitate was counted by an automatic model 5240 Packard gamma-spectrometer, which has an effectiveness

of ^{125}I counting equal to S4 percent. When nonspecific radioactivity was too high, greater than 10 percent, the precipitate was rinsed one to three times with RIA buffer, and readings were taken once again.

The results were given as the percent of radioactivity bound in the immune complex in the presence of the competitor, with respect to the bound radioactivity of one labeled standard (^{125}I-hemagglutinin).

Results and Discussion

We adhered to the following principle when performing quantitative radioimmunological analysis: A constant quantity of antihemagglutinating serum and a constant quantity of labeled hemagglutinin were allowed to react with a variable quantity of unlabeled hemagglutinin (standard) or the sample under analysis, in which case competition for binding points on antibodies occurs between unlabeled and labeled antigen.

To permit quantitative assessment of the result, the concentration of hemagglutinin in the material under analysis was determined in parallel with a standard analysis , in which the dependence, in relation to dose, of competition between initial unlabeled hemagglutinin and hemagglutinin in the test system was revealed. This dependence is shown in Figure 1 either as an exponential curve when the percent label binding is plotted on the ordinate axis and the dose, in nanograms, of competing unlabeled hemagglutinin is plotted on the abscissa on a linear scale, or as a straight-line segment of the dose curve when the percent label binding is given as a $\log_{10}$ function of the dose of unlabeled hemagglutinin. As we can see from Figure 1, the method allows us to reveal down to 1 nanogram of the tested antigen in a sample. Higher sensitivity of the system would require an increase in the specific activity of labeled hemagglutinin. However, despite the fact that the *in vitro* iodination method basically allows us to obtain ^{125}I-hemagglutinin preparations containing a very high concentration of ^{125}I, our experience showed that immunological specificity in such preparations is significantly low or entirely lost, and that ^{125}I-hemagglutinin preparations with specific activity not exceeding 3 μKi/μg are optimum for radioimmunological analysis. In this aspect our research agrees with recently published data (2).

The specificity of the method was assessed by comparative study of the capability of antiserum in a 1:15,000 working dilution for binding with a fixed quantity of ^{125}I-hemagglutinin in the presence of different doses of the following competing agents: Unlabeled H3 hemagglutinin as the standard; purified and concentrated influenza virus A/Port-Chalmers/1/73 (H3N2) and influenza virus A/MRC-11 (H3N2) in the form of allantoic fluid; purified and concentrated influenza virus A/PR8 containing antigenically distinctive HO hemagglutinin.

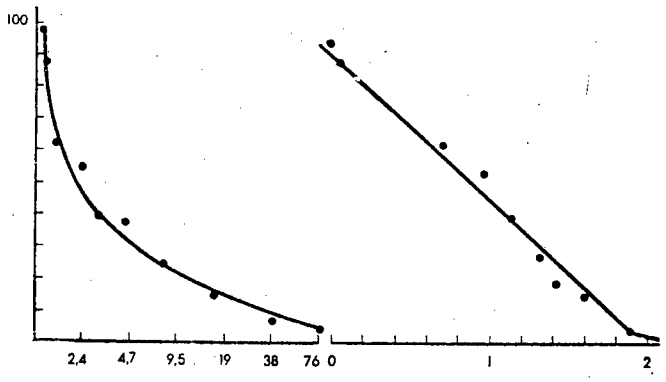

Figure 1. Standard Curve Showing the Dependence of H3 ^{125}I-hemagglutinin Binding on the Dose of Unlabeled H3 Hemagglutinin: Ordinate--percent H3 ^{125}I-hemagglutinin binding; abscissa--dose of unlabeled H3 hemagglutinin in nanograms (in linear units on the left, in logarithmic units on the right).

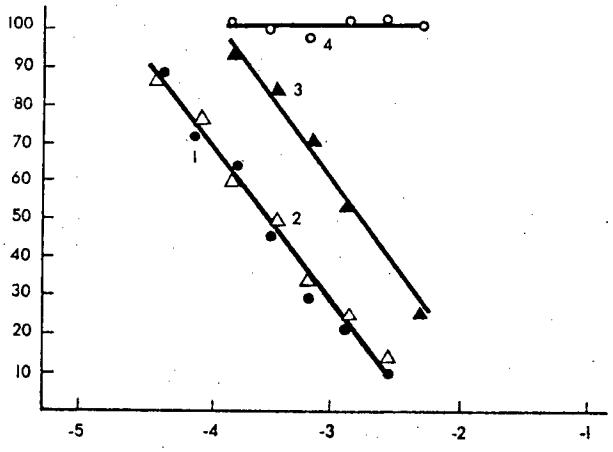

Figure 2. Dependence of Binding of H3 ^{125}I-hemagglutinin into an Immune Complex on the Doses of Various Competing Agents: *1*--H3 hemagglutinin; *2*--purified concentrated influenza virus A/Port-Chalmers/1/73 (H3N2); *3*--allantoic fluid containing influenza virus A/MRC-11 (H3N2); *4*--influenza virus A/PR8 (H0N1); Ordinate--percent binding of H3 ^{125}I-hemagglutinin; Abscissa----logorithm of the dilution of the competing agents.

We can see from Figure 2 that the percent binding of ^{125}I-hemagglutinin into an immune complex declined regressively as the quantity of influenza virus A/Port-Chalmers?1/73 increased. This indicates that its antigenic determinants compete with H3 hemagglutinin for antibodies. In this case the points on the dose curve of competition superimpose over the standard curve, which indicated that antigenically it is totally identical to H3 hemagglutinin. Influenza virus A/MRC-11 from the same subgroup (H3N2) also exhibited antigenic kinship (the dose dependence curves are parallel), but its H3 hemagglutinin concentration is lower, and a larger quantity of material would be needed to achieve equivalent competition with ^{125}I-hemagglutinin.

A/PR8 influenza virus containing hemagglutinin in a different antigenic group, H0, exhibited competition in the entire range of doses.

The experimental results graphically show that competitive radioimmunological analysis is specific enough to differentiate influenza virus strains with respect to the antigenic determinants of hemagglutinin.

Discussing the specificity of RIA, however, we must consider two important factors. First, the main factor defining the method's specificity is high immunological "purity" of the test system, mainly of the standard antigen, which must be chemically and antigenically homogeneous. We tried to use influenza virus labeled with ^{125}I in the test system. But even when high-quality antihemagglutinating serum was employed, nonspecific binding of the label increased by several orders of magnitude as compared with the level attained with use of hemagglutinin as the test antigen. Second, no matter what virus preparations are used in the RIA, we should avoid work with undiluted biological material, particularly with undiluted virus-containing allantoic fluid, since our experience showed that the nature of this dose dependence changes in extreme conditions, and we would risk a false positive result of competition, probably due to the blockage of the active centers of antibodies by a large quantity of impurities present in the allantoic fluid.

Assessing the sensitivity or RIA in determining hemagglutinin concentration, using, as our basis, agreement of the dose curves of competition between influenza virus A/Port-Chalmers?1/73 and the standard with a known quantity of H3 hemagglutinin measured precisely by Lowry's method (see Figure 2), we can graphically compute the hemagglutinin concentration in weight units for each dilution of influenza virus. Simultaneous titration in the hemagglutination inhibition test permits us to compare the sensitivities of the two methods for determining hemagglutinin concentration.

The data and the table on the following page graphically show that the sensitivity of the method under investigation here is significantly higher than that of the hemagglutination inhibition test: We were able to reveal fully definite quantities of hemagglutinin by the RIA method at influenza virus dilutions of 1:100, 1:250, and 1:500, at which negative results were obtained with the hemagglutination inhibition test.

Comparative Assessment of the Sensitivity of Determining Hemagglutinin in Influenza Virus A/Port-Chalmers/1/73 Using the Hemagglutination Inhibition Test and the RIA Method.

(1) Разведение	РГА (2) (4) титр ГА в 0,2 мл	РИА (3) (5) количество ГА, нг/0,2 мл
1:2	1:512	>300
1:5	1:32	150
1:25	1:4	113
1:50	1:2	21,5
1:100	0	13,5
1:250	0	6,5
1:500	0	3,8

NOTE: The numerical data in the table show the quantity of H3 hemagglutinin in the test system to which the hemagglutinin concentration in the analyzed sample is equivalent.

Key:

1. Dilution
2. Hemagglutination inhibition test
3. RIA
4. Hemagglutinin titer in 0.2 ml
5. Hemagglutinin quantity, nanograms/0.2 ml

Analyzing the data of our research, we can conclude that competitive radioimmunological analysis can be used to characterize the antigenic specificity of influenza virus hemagglutinin and, on an extensive scale, to titrate influenza virus strains. Use of a test system consisting of highly purified, antigenically homogeneous H3 hemagglutinin as the standard and highly specific antiserum significantly increases the method's specificity and makes it promising for quantitative determination of changes in antigenic determinants within a single influenza virus subgroup.

The test system we developed was used successfully to study antigenic drift of epidemically active human influenza viruses (H3N2). The results of this research will be presented in the next communication.

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INVESTIGATION OF FOAMY VIRUSES OF BABOONS AND MACAQUES

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 179-187

[Article by V. Z. Agrba, L V. Kokosha, L. A. Yakovleva, V. V. Timanovskaya, and G. N. Chuvirov, Institute of Experimental Pathology and Therapy, USSR Academy of Medical Sciences, Sukhumi]

[Text] The detection frequency of foamy viruses among healthy baboons (*Papio hamadryas*) and macaques (*Macaca arctoides* and *Macaca cynomolgus*), hemoblastotic monkeys, and ones inoculated with leukemic materials was investigated. The behavior of viruses in cultured animal cells and in some other sensitive cell systems is described. An electron microscopic description is given of foamy viruses with dimensions of 90-100 nm, an opticoelectronically light nucleoid with dimensions of 40-50 nm, and processess on the capsid 10-12 nm long, a characteristic trait of foamy viruses. The isolated viruses were basically identified as serological type 2 on the basis of neutralization and immunofluorescent reactions. Data are also presented indicating presence of serological type 1 virus. The percentage of virus isolated increases with animal age, attaining 88.8-100 percent among adult specimens. The placenta acts to some extent as a barrier to foamy viruses, since in the examined cases foamy viruses were not isolated from embryonic tissues.

Our virological investigation of experimental hemoblastosis in monkeys of two species--baboons and macaques, particularly when we cultured blood cells and cells from hemopoietic organs, revealed an agent eliciting a cytopathic effect recalling the action of foamy viruses.

Works discussing isolation and investigation of foamy viruses from monkeys began to appear in the 1960's in connection with growth in the use of primates and cell cultures obtained from them in experimental biology and medicine (1-5).

There are now eight types of monkey foamy viruses. Types 1-5 were isolated from lower primates (6-13), while types 6 and 7 were isolated from chimpanzees (4,14,15). A foamy virus antigenically different from known type 1-7 viruses has been isolated from the tissues (liver, spleen, cerebral cortex) of spider monkeys (16).

We had reported detection of foamy viruses in the tissues of *Papio hamadryas* striken with hemoblastosis earlier (17). The present communication provides data on the occurrence of foamy viruses among healthy monkeys, hemoblastotic monkeys, and ones inoculated with leukemic materials; the serological identification of this virus is presented as well.

Materials and Methods

Culturing procedures: Cells from hemopoietic organs (bone marrow, spleen, lymph nodes, leukocytes) were cultured in RPMl-1640 medium combined with 20 percent bovine embryonic serum inactivated by heating. The cell cultures were incubated at 37°C in an air-gas mixture consisting of 5 percent CO_2 and 95 percent air. Cells from monkey hemopoietic organs were cultured together with innoculated cultures of dog thymocytes (FCf_2Th), mink lung cells (MvlLu), and rabbit corneal cells (SIRC). Preliminarily trypsinated human embryonic tissue (HET) and a 1-day rabbit kidney cell (RK) cultures were used to titrate and identify the virus. These cell cultures were obtained in the laboratory and maintained in an MEM medium together with 2-10 percent calf serum inactivated by heating. Cover glasses bearing grown cell culture were rinsed to remove serum, fixed with methanol for 20-30 minutes, and then stained with hematoxylin-eosin.

Serology: Neutralization and immunofluorescence reactions were used to identify the virus. Standard equine antiserum to type 1, 2, and 4 foamy viruses, which are encountered most frequently among lower primates, were used as immune sera. The neutralization reaction was performed with RK cell culture. A 100(TTsD)/1.0 ml dose of the virus was mixed with an equal volume of immune serum heated at 56°C for 30 minutes. Immune serum was used at dilutions of 1:10-1:20. The mixture was incubated at 37°C for 60 minutes. Then this mixture was placed in four flasks with cover glasses bearing a 1-day RK cell culture. The flasks were incubated at 37°C for 14 days. The results were recorded on the 9th day, when complete destruction of the cell layer was observed in control flasks infected with 100 TTsD of the virus.

Virus antigen was revealed by an indirect immunofluorescence test. Tissue culture cells were fixed by cooling at 4°C with acetone for 10 minutes. Immune sera at a 1:40 dilution were used in the reaction. Antiserum to equine globulins labeled with (FITTs) were used at a dilution of 1:16. Immune and FITTs-labeled sera were treated for 30 minutes at 37°C.

Electron microscopy: Cells were fixed with 2 percent glutaric aldehyde, rinsed with phosphate buffer, and subjected to final fixing with 1 percent OsO_4. Dehydration was performed in acetone of rising concentration. Then the cells were placed in epon-812 epoxy resin. Ultrathin sections were obtained with an LKB microtome and contrasted with uranyl acetate and lead citrate. The material was studied with a JEM-100u electron microscope at an instrumental magnification of 5,000-30,000×.

Results

Baboons (*Papio hamadryas*) and macaques (*M. arctoides* and *M. cynomolgus*) were used in the experiments--healthy monkeys, monkeys inoculated with leukemic materials, and monkeys afflicted by hemoblastosis. Attachment and growth of cells on the glass surface were uneventful in all cases at the beginning of the culturing procedure. A monolayer consisting basically of fibroblast-like cells formed after a week of culturing. Singular giant cells containing a large quantity of nuclei appeared on the background of the smooth monolayer in 16-23 days as a rule, after one or two subinoculations. These cells did not have distinct perimeters, and their shape was odd. Then the pathological pattern developed quickly, expressing itself as arisal of spotty vacuolization in cytoplasm of these giant cells. When Giemsa-Romanovsky staining was employed, symplasts with nuclei attaining quantities of 50 and higher could be seen in preparations of these cultures (Figure 1). The monolayer assumed a clearly expressed "openwork" appearance 2-3 days following appearance of the giant cells. A large quantity of large floating cells, often vacuolated, and their accumulations were noted in the liquid phase.

A similar phenomenon occurred in indicator cell cultures (FCf_2Th, MvlLu, SIRC) 8-20 days after joint culturing with cells from monkey hemopoietic organs, with arisal and intensification of the foaming effect proceeding in parallel with cell proliferation. When the cell proliferation index was artificially reduced, arisal of foamy degeneration was retarded. As a rule complete vacuolization and destruction of the cell layer occurred 5-7 days following the occurence of the first signs.

As is shown in Table 1, foamy viruses are isolated from all hemopoietic organs of both species of adult monkeys. These viruses are detected with identical constancy in the organs of hemoblastotic monkeys, monkeys inoculated with leukemic materials, and healthy animals.

In our experiments we used embryonic tissue from monkeys in the second half of pregnancy. Foamy viruses were not isolated from any of eight embryonic tissue cultures (four from baboons and four from macaques). In two cases we studied the placenta and embryo of the same animal (*M. cynomolgus*). In both cases we isolated viruses from the placenta, while signs of the presence of foamy viruses were not revealed in embryonic tissue in a long period of observation (up to 20 subpopulations). Virus was isolated in one

Table 1. Frequency of Isolation of Foamy Viruses from Monkey Tissues.

(1) Вид обезьян	(2) Характеристика животных	(3) Число обезьян	(4) Частота выделения вируса из тканей обезьяны						
			(5) взрослых					(6) эмбрионов	
			селезенка (7)	костный мозг (8)	лейкоциты (9)	лимфатический узел (10)	плацента (11)	КМ	КМТ
Papio hamadryas	(12) Здоровые	4	1/1	2/2	—	—	—	0/2	0/2
	(13) Больные гемобластозом	14	6/7	6/7	—	1/2	—	0/2	0/2
M. arctoides	(14) Инокулированные	10	—	10/10	10/10	—	—	—	—
	(13) Больные гемобластозом	7	5/5	5/5	—	—	—	0/2	—
M. cynomolgus	(14) Инокулированные	2	—	—	—	—	2/2	0/2	0/2

Note: KM--embryonic bone marrow culture; KMT--embryonic cutaneomuscular cell culture. Here and in Table 2: Numerator--number of times foamy viruses were isolated; denominator--number of monkeys examined.

Key:

1. Monkey species
2. Animal characteristics
3. Number of monkeys
4. Frequency of isolation of virus from monkey tissues
5. Adults
6. Embryos
7. Spleen
8. Bone marrow
9. Leukocytes
10. Lymph node
11. Placenta
12. Healthy
13. Hemoblastotic
14. Inoculated

case from the tissue of a 1 day old infant. Percent isolation of viruses increases with the animal's age, attaining 88.8-100 percent for the adult monkeys examined (Table 2).

To collect a yield of the virus at the peak of the pathological course, we inoculated preliminarily trypsinated HET and RK cell cultures with cells resuspended in the liquid phase. Initiation of cytopathic action in HET and RK cells was noted 5-7 days after infection, and complete destruction of the monolayer was observed after 9-12 days. The moment of appearance of sick fibroblasts, sometimes with a roughly outlined membrane, was interpreted as the beginning of specific degeneration in HET cell culture. The outlines of some of the cells were diffuse (Figure 2). Multinucleated cells with vacuolated cytoplasm were encountered rather often. As the observations proceeded, the pathological pattern increased in intensity, expressing itself as lysis of most cells by the 9th-14th days after infection. All virus materials obtained from different animals produced a similar pattern in HET

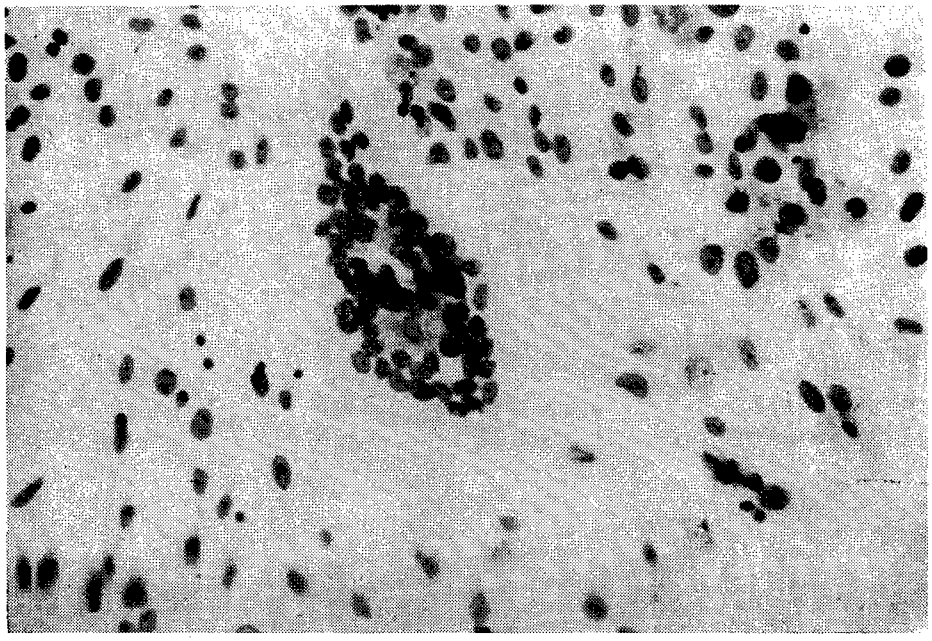

Figure 1. Mononuclear Cell in a Culture of Baboon Bone Marrow Cells: Here and in figures 2-5: Giemsa-Romanovsky staining; magnification 200×.

and RK cells (Figure 3), differing dramatically from the appearance of uninfected cells (Figure 4).

Electron microscopic investigation of primary culture cells and virus-infected cultures revealed a virus having morphological characterisitcs related to the foamy virus group. Mature virus particles with a diameter of 90-100 nm had an opticoelectronically light nucleoid with dimensions 40-50 nm and growths on the capsid 10-12 nm long (Figure 5). The latter are a typical sign of foamy viruses. Mature virions were encountered in intercellular space and in channels of the endoplasmic reticulum. Particles budding from the plasma membrane of the cells and from membranes in the endoplasmic reticulum were ofter encountered. As a rule the budding particles possessed a formed nucleoid and growths on the surface.

In the neutralization reaction we noted total inhibition of the cytopathic action of the virus in flasks containing a mixture of the virus and antiserum to type 2 foamy virus. At the same time a slight delay in destruction of the cell layer was noted in some cases when antiserum to type 1 foamy virus was employed. As with physiological solution and normal rabbit and guinea pig sera, antiserum to type 4 foamy virus did not prevent complete destruction of the cell layer.

Table 2. Frequency of Isolation of Foamy Viruses Depending on Host Age.

(1) Вид обезьян	(2) Возраст			
	(3) эмбрион	(4) 1 день	(5) 1—5 лет	(6) 5 лет и старше
(7) Павианы гамадрилы	0/4 (0)	1/1 (100)	3/4 (75)	8.9 (88,8)
(8) Макаки	0/4 (0)	— —	17/17 (100)	— —

Note: Percentage is shown in parentheses.

Key:

1. Monkey species
2. Age
3. Embryo
4. 1 day
5. 1-5 years
6. 5 years and over
7. *Papio hamadryas*
8. Macaques

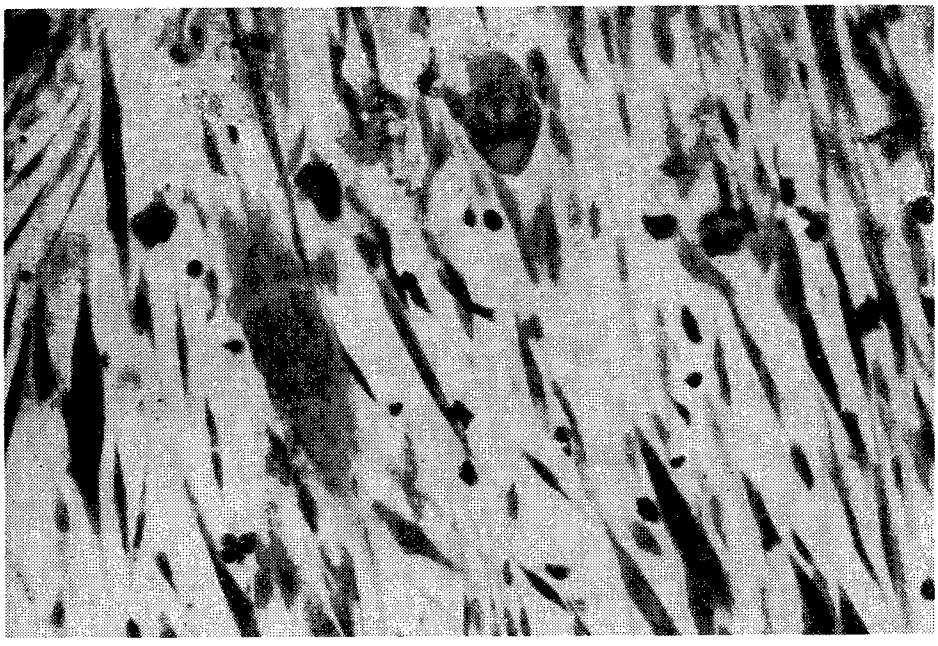

Figure 2. Degeneration in Human Embryonic Cell Culture Caused by Foamy Virus.

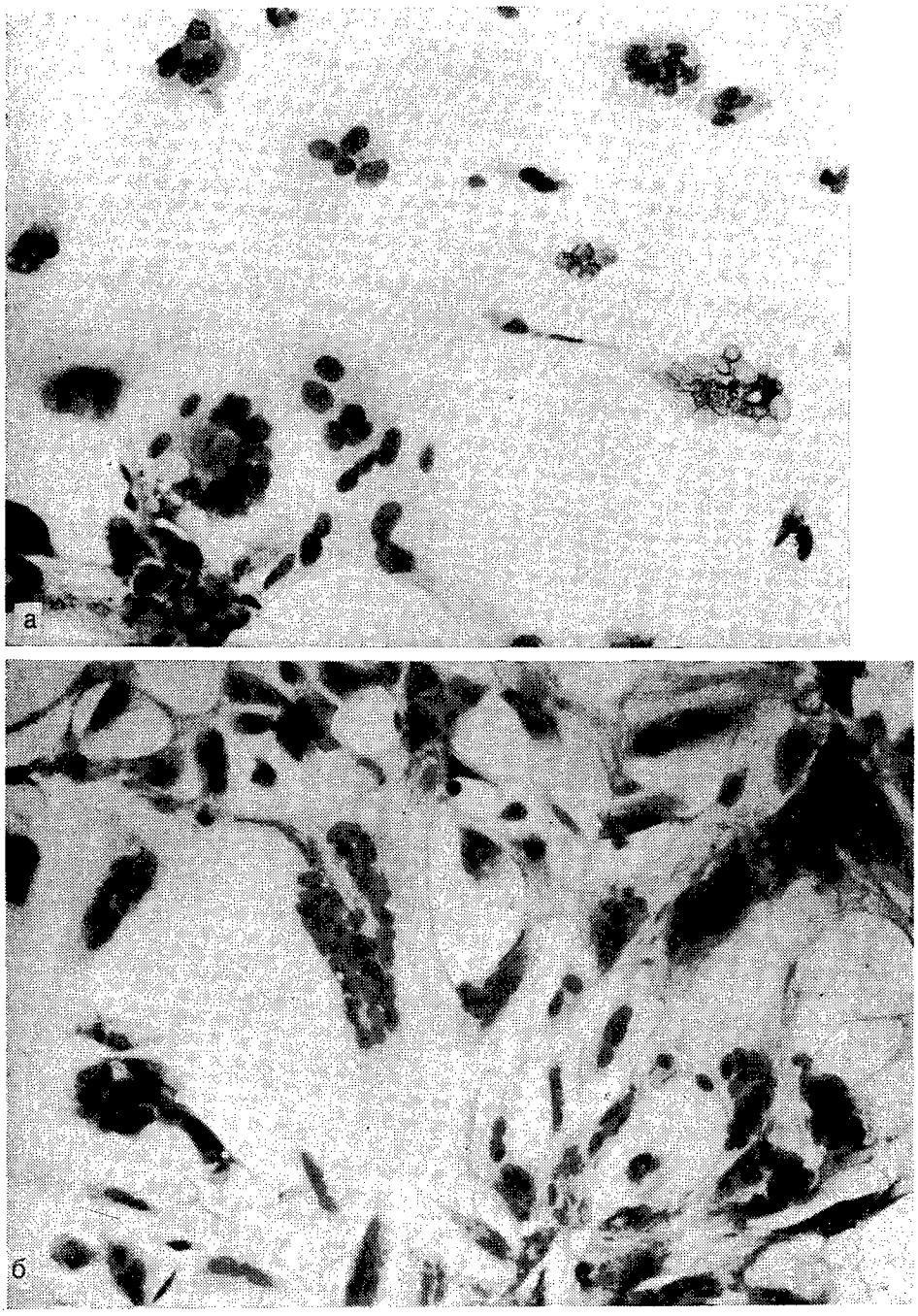

Figure 3. Degeneration in Rabbit Kidney Cell Culture Caused by Foamy Viruses Isolated from Different Monkeys (*a*,*b*).

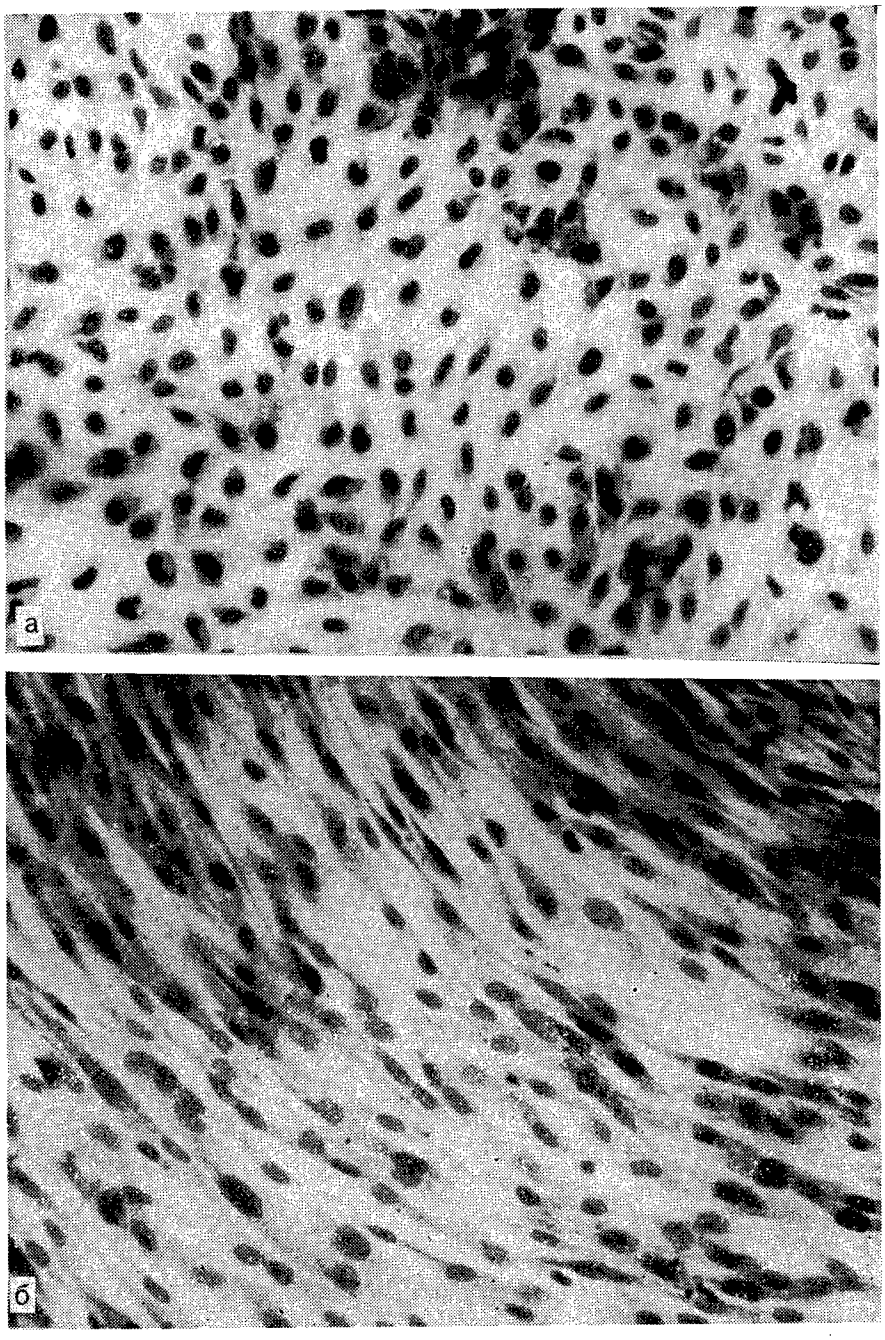

Figure 4. Appearance of Cells in Uninfected Human Embryo (*a*) and Rabbit Kidney Cell Cultures (*b*).

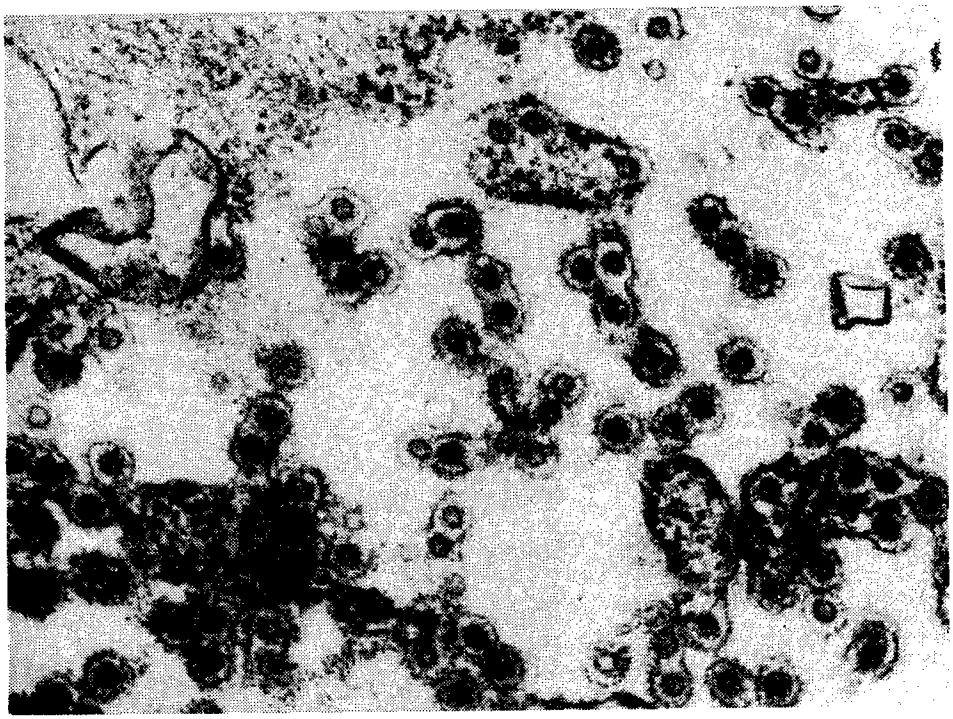

Figure 5. Foamy Viruses in Cultured Monkey Cells: Magnification 70,000×.

Immunofluorescence revealed mononucleate cells with a bright fluorescent cytoplasm in primary culture cells treated with antiserum to type 1 and 2 foamy viruses. We did not reveal fluorescent cells in the same cultures treated with antiserum to type 4 foamy virus, physiological solution, or normal serum. Analysis of cell cultures sensitive to foamy viruses (HET, RK) 12 days following infection by virus-containing material showed that antiserum to type 2 foamy virus produces 78-94 percent cells with bright cytoplasmic fluorescence, while antiserum to type 1 foamy virus produces only 14-28 percent cells with a similar reaction. Analysis of HET cell cultures infected with foamy virus revealed, for the first time, virus antigens 30 hours after infection. At first the antigen was detected in the nucleus and cytoplasm of a small number of cells, while after 93 hours bright cytoplasmic fluorescence was noted in 92 percent of the cells.

Discussion

Judging from its behavior in cell cultures in the investigated monkeys and in processed sensitive cell systems, as well as from the morphological characteristics determined by electron microscopic analysis, the agent we investigated is related to the foamy viruses (2,5,7,8,18-21). The animals we investigated were basically carriers of type 2 foamy virus, which was identified in neutralization and immunofluorescence reactions. At the same time it could be hypothesized that type 1 foamy virus is also present in some animals. A similar phenomenon has already been described in the literature (8), where type 1 foamy virus was revealed in 89 out of 119 isolations of foamy virus from macaque kidney cultures and type 2 foamy virus was isolated in 12 cases. The authors explain this fact by cross-contamination of monkeys of different species maintained in quarantine.

This is the first time research of this sort has been conducted on *Papio hamadryas*, though some communications (8,13) report isolation of foamy viruses from baboons or an unidentified species, or from *Papio cynocefalus*.

We know from the properties of foamy viruses that they do not elicit pathology in small laboratory animals, that they do not have a capacity for hemadsorption, that they do not form hemagglutinin, and that they do not produce intranuclear and intracytoplasmic inclusions sensitive to chloroform and an acid medium (pH 3.0). Foamy viruses possess revertase activity, and their buoyant density in sucrose solution is about 1.16 gm/cm^3 (5,6, 15,21,22). The morphogenesis of foamy viruses has been studied (7,14,18, 20,23).

The high percentage (88.8-100) of persistence of foamy viruses in the hemopoietic organs of adult baboons and macaques of the Sukhumi herd agrees with the data of other authors (8), who have detected type 1 and 2 foamy viruses in the spleens of 86 percent of the macaques examined. In addition, serological research conducted on chimpanzees indicated extremely high persistence (91-100 percent) of foamy viruses in the bodies of these animals (15).

Our research demonstrated that the placenta is a good barrier to foamy viruses, since viruses were not detected in any of eight observations of embryonic cell cultures. Moreover, while foamy virus was isolated from the placentas of two monkeys, the virus was not detected in embryonic tissue. Nevertheless the placenta's barrier function in relation to foamy viruses is not absolute, since in one case virus was isolated from the body of a 1 day old infant which had obviously acquired the virus while within the womb.

The role of foamy viruses in the organism is unclear (24). Clarke et al., (25) interpret them as endosymbiotic viruses. Other authors (13) suggest that the viral genome is incorporated in cellular DNA, and that this possibly explains intensification of cytopathic action as cell culturing time increases. Foamy viruses are widespread in nature. They have been isolated from human tumors (19) and from cattle and cats (21,26-28). Monkeys

of different species, including chimpanzees, macaques, green marmosets, tamarins, spider monkeys, lemurs, and baboons are carriers of foamy viruses (9,13,16,29-31). Our data supplement existing information on the widespread occurrence of foamy viruses in monkeys of different species.

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REVERSION IN NATURAL VARIABILITY OF TYPE A INFLUENZA VIRUS

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 146-151

[Article by M. A. Yakhno, V. A. Isachenko, Ye. V Molibog, S. S. Yamnikova, G. K. Vorkunova, O. N. Berezina, V. T. Ivanova, N. V. Klitsunova, G. G. Khokhlova, N. V. Antonova, D. Ya. Zakstel'skaya, and V. M. Zhdanov, Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow]

[Text] In mid-November 1977, local outbreaks of acute respiratory diseases (ARD) began to be recorded in organized collectives in a number of geographical zones of the USSR. A general increase in morbidity was observed in certain regions of the country by as early as the end of the month. The epidemic outbreaks spread gradually, and they were typified by moderate development mainly involving young persons.

The strains causing the epidemic had no antigenic relationship to the standard A(H3N2) strain, and they were inhibited at up to 1.25 titer by antisera to type A influenza virus with the antigenic formula H1N1. Their neuraminidase was inhibited by antiserum to recombinants containing subtype N1 neuraminidase. Analysis of polypeptides isolated from the strains by electrophoresis in polyacrilamide gel revealed identity of the main structural proteins with those of standard virus A/FM/1/47, with the exception of a heavy hemagglutinin chain.

The data permit classification of the strains causing the epidemic outbreaks in November-December 1977 as type A influenza virus with an antigenic formula H1N1.

Observations made by the Regional Influenza Center over a period of many years indicate that influenza epidemics are associated with arisal of influenza virus strains with a new antigenic formula (1). Prompt

determination of the antigenic structure of strains causing both local outbreaks and epidemics acquires especially important significance in this connection. The present communication provides data on the antigenic characteristics of influenza viruses that had caused a series of local outbreaks in November-December 1977 on USSR territory.

Materials and Methods

The viruses: Fifty-four hemagglutinating agents isolated in November-December 1977 during an influenza outbreak in Moscow, Khabarovsk, Vladivostok, Novosibirsk, Belgorod, and Poltava were studied. The first strains reached the Regional Center on 21 November 1977 from the virological laboratory of the Moscow City Epidemiological Station (A. M. Osherovich and Ye. P. Neyevina) and on 28 November from the laboratory of viral infections and clinical immunology of Institute of Virology imeni D. I. Ivanovskiy (Prof Ye. S. Ketiladze and Doctor of Medical Sciences S. A. Demidova) without being identified. Strains reached the Center on 30 November 1977 from the virology laboratory of the Khavarovskiy Kray Epidemiological Station (V. I. Reznik), and on 7 December 1977 from the Vladivostok Institute of Epidemiology, Microbiology, and Hygiene (Director--Candidate of Medical Sciences, T. V. Pysina), initially titrated as A1 using the hemagglutination inhibition test.

The sera: We used diagnostic sera produced by the Leningrad Institute of Vaccines and Sera, WHO sera, and sera prepared by the authors using rabbits and rats effective against the following standard strains of human influenza virus: A/PR8/34/(HONl), A/WS/33/(HONl), A/FM/1/47(HlNl), A/Klim/49(HlNl), A/Pan/52(HlNl), A/Singapore/1/57(H2N2), A/Hong Kong/8/68(H3N2), A/Port-Chalmers/1/73(H3N2), A/Victoria/3/75(H3N2), A/Texas/1/77(H3N2), and B/Hong Kong/7/75, and animal influenza viruses: A/FPV/27(HavlNeql), A/chick/Germany/"N"/49(Hav2Nl), A/duck/England/56(Hav3Navl), A/duck/Czechoslovakia/56(Hav4Navl), A/tern/South Africa/61(Hav5Nav2), A/turkey /Massachusetts/65(Hav6N2), and A/duck/Ukraine/1/63(Hav7Neq2).

In addition we used antisera to recombinants containing hemagglutinin Heql and neuraminidase Nl, N2 from strains A/FM/1/47, A/Hong Kong/68, and A/Victoria/3/75. We also used antisera to purify hemagglutinins of human and animal type A influenza virus subtypes graciously provided by Dr Webster, and antiserum to pure hemagglutinin from strain A/Port-Chalmers/1/73 obtained by a method described earlier (2). The sera were treated with RDE to remove inhibitors.

The serological test: The double gel precipitation test was performed by the method of Schild and Pereira (3) using antiserum to ribonucleoprotein (RNP) from strain A/Port-Chalmers/1/73.

The hemagglutination inhibition test (HIT) was performed with 4 antigenic units of antigen and a 1 percent suspension of chick embryo erythrocytes.

The neuraminidase activity extinction test (NAET) was performed in accordance with a universally accepted WHO procedure (4) using fetuin as the substrate.

The method used for radioimmunological analysis (RIA) was described earlier (5). Purified hemagglutinin from type H3(MRC-11) influenza virus and its specific antiserum were used as the test system.

Native allantoic cultures were analyzed with an electron microscope by the negative contrast method.

Statistical data concerning influenza and ARD morbidity were obtained from research bases of the USSR Regional Center.

Results

Small influenza outbreaks began to be recorded in mid-November 1977 in a number of cities located in different geographical zones of the country, especially in young collectives. Epidemic growth of influenza morbidity began in the last third of November in Vladivostok, Khabarovsk, and Novosibirsk. By the end of November and beginning of December 1977, the influenza epidemic had spread to Leningrad, Murmansk, Moscow, Poltava, and other cities located in different parts of the country. The start of the epidemic was characterized by a dramatic increase in morbidity.

Preliminary analysis of viruses isolated during the outbreaks showed that they do not interact with antisera to A(H3N2) strains which had been circulating since 1968, to include antisera to A/Victoria/3/75 and A/Texas /1/77. In the double gel precipitation test, concentrated preparations from the new strains formed distinct precipitation lines with antiserum to purified RNP from type A influenza virus and, consequently, they were of this type (Figure 1).

Analysis of eight strains in the preparations with an electron microscope regularly revealed virions typical of influenza virus, with filiform form with a cross section of 80-90 nm and a length of up to 1-1.5 μ (Figure 2).

An HIT was performed to determine specificity of hemagglutinin: A set of antisera to four known subtypes of human hemagglutinin was used to analyze 54 isolates from different localities; the five most typical strains were additionally analyzed with a set of antisera to hemagglutinin of human and animal influenza viruses. Not one of the 54 analyzed isolates reacted with antisera to strains possessing hemagglutinins H3, H2, or H0. At the same time all 54 analyzed strains were clearly inhibited

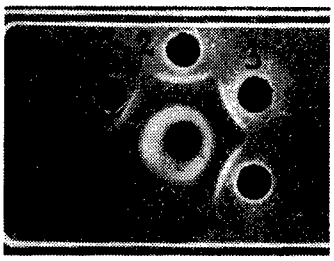

Figure 1. Gel Precipitation Reaction of Concentrates from Newly Isolated Strains Using Type A Influenza Virus Ribonucleoprotein Antiserum: Central craters contain serum; peripheral craters contain viral antigens in the following order: *1*--Khabarovsk/034574/77; *2*--Moscow /0897/77; *3*--Moscow/0675/77; *4*--A/Port-Chalmers/73; *5*--B/Li/40; *6*--Negative control

by antisera to strains having hemagglutinin H1. In this case hemagglutination of newly isolated strains was inhibited both by WHO antiserum to strain FM/1 to full or one-fourth homologous titer, and by diagnostic serum produced by the Leningrad Institute of Vaccines and Sera to the full or double titer (Table 1), as well as by antiserum to pure hemagglutinin A (H1), obtained from Dr Webster, to the full titer (Table 2). Not one of the antisera to strains having hemagglutinins typical of horse, swine, or avian influenza viruses had an inhibitory effect on any of the five analyzed strains (see Table 2). Eight additionally analyzed isolates were inhibited by antisera to the domestic strains A/Pan/52 and A/Klim/49 possessing hemagglutinin H1. Thus the hemagglutinins of all newly isolated strains analyzed by the standard test are subtype H1.

In the next stage of our research we established that neuraminidase of all analyzed strains was inhibited best of all by antisera to subtype N1 (Table 3). In this case some isolates were inhibited actively by antisera to some strains having subtype N1, including A/PR8/34, A/FM/1/47, and A/New Jersey/8/76, while in other isolates neuraminidase exhibited a relationship to subtype N1 on the basis of one or two of the antisera used (see Table 3).

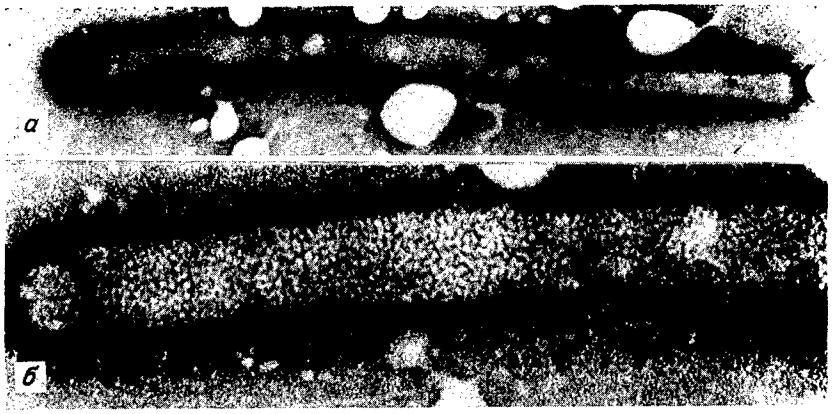

Figure 2. Filiform Influenza Virus Virions Discovered in Isolate 0782: Negative staining with 1 percent uranyl acetate solution; magnification 60,000× *(a)*, 180,000× *(b)*.

In addition among the total number of analyzed strains we revealed viruses for which neuraminidase was inhibited by, in addition to antisera to subtype N1, antisera to subtype N2, though to a lesser degree. Perhaps this reflects heterogeneity of the population owing to a combination of previously circulating and newly appearing viruses. However, this problem requires special research.

It should be emphasized that heterogeneity of strains was also noted in their analysis by the RIA method using a test system consisting of purified H3 hemagglutinin and antiserum to it. Similarly as with standard strains A (H1N1) and A (H0N1), some of the strains did not compete with labeled H3 antigen for binding points, indicating absence of the corresponding antigenic determinants. Other strains did reveal a capability for different degrees of competition with labeled H3 antigen.

Polypeptides from the isolated strains and from FM/1/47, a strain that had been circulating in 1947-1957, were compared by electrophoresis in 10 percent polyacrilamide gel according to Laemmli's method (10). Figure 3 shows the stained laminar gels used to analyze polypeptides from marker viruses A/Port-Chalmers (gel *A*,*1* and *10*) and FM/1/47 (gel *A*, *2* and *9*; gel *B*, *1* and *5*), and from epidemic strains isolated in Moscow and Khabarovsk. The following polypeptides are evident in the composition of the marker viruses: P (from one to three proteins), hemagglutinin precursor HA, nucleocapsid protein NP, heavy hemagglutinin chain HA_1, light hemagglutinin chain HA_2, membrane protein M. HA_2 follows after M in strain FM/1/47. Polypeptides from all three epidemic strains are identical to each other, and most of them (HA, NP, M, and HA_2) migrate in the same way as do the corresponding FM/1 polypeptides. The main differences involved HA_1 protein

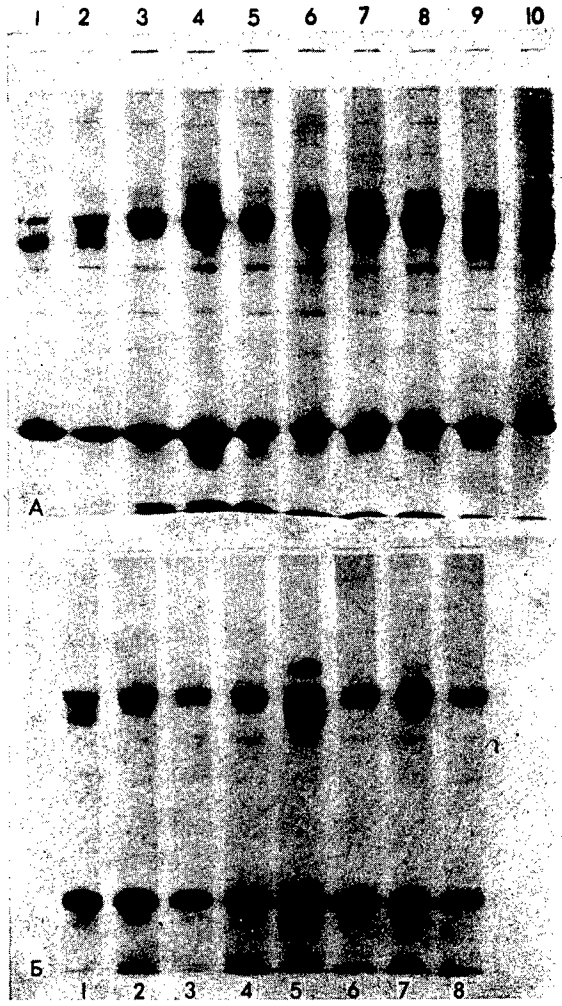

Figure 3. Laminar 10 percent Polyacrilamide Gels, Stained With Amido Black, Following Electrophoresis of Polypeptides from Purified Viruses: *A*--Port-Chalmers/73, FM/1/47, Moscow/0897/77, Moscow/0782/77, Khabarovosk/034561/77 and, treated with urea, Khabarovosk/034561/77, Moscow/0782/77, Moscow/0897/77, FM/1/47, Port-Chalmers/73; *B*--FM/1/47, Moscow/0897/77, Moscow/0782/77, Khabarovosk/034561/77, FM/1/47, Moscow/0897/77, Moscow/0782/77, Khavarovsk/034561/77

(1) Место изоляции штамма	(2) Число изученных штаммов	(3) Сыворотки к штаммам											
		A/PR8/34 (H0N1) 1:160*	A/WS/33 (H0N1) 1:1280	FM/1/47** 1:320		A1*** 1:160		(6) A/Сингапур/1/57 (H2N2) 1:1280	(7) A/Гонконг/8/68 (H3N2) 1:5120	(8) A/Порт-Чалмерс/1/73 (H3N2) 1:1280	(9) A/Виктория/3/75 (H3N2) 1:640	(10) A/Техас/1/77 (H3N2) 1:1280	(7) B/Гонконг/7/75 1:1280
				(4) число положительных	(5) титр ингибиции (обратная величина)	(4) число положительных	(5) титр ингибиции (обратная величина)						
(11) Москва	8	0	0	8	80—160	8	320—640	0	0	0	0	0	0
(12) Хабаровск	18	0	0	18	80—160	18	160—640	0	0	0	0	0	0
(13) Новосибирск	10	0	0	10	80—160	10	160—320	0	0	0	0	0	0
(14) Владивосток	5	0	0	5	80—160	5	320—640	0	0	0	0	0	0
(15) Белгород	6	0	0	6	160—320	6	320—640	0	0	0	0	0	0
(16) Полтава	7	0	0	7	80—160	7	320—640	0	0	0	0	0	0
(17) Итого . . .	54												

Table 1. Hemagglutination Inhibition of Newly Isolated Strains by Standard Antisera

*Homologous titers of standard sera
**Standard serum was obtained from WHO
***Commercial serum prepared by the Leningrad Institute of Vaccines and Sera; the titer indicated on the label is given

Key:

1. Place of isolation of strain
2. Number of strains studied
3. Antisera for the following strains
4. Number positive
5. Inhibition titer (reciprocal)
6. Singapore
7. Hong Kong
8. Port-Chalmers
9. Victoria
10. Texas
11. Moscow
12. Khabarovsk
13. Novosibirsk
14. Vladivostok
15. Belgorod
16. Poltava
17. Total

(1) Антигены	Сыворотки (2)													
	H0	H1	H2	H3	Heq1	Heq2	Hav1	Hav2	Hav3	Hav4	Hav5	Hav6	Hav7	$H_{SW}1$
A/PR/8/34 (H0N1)	2560	160	0	0	0	0	0	0	0	0	0	0	0	0
A/FM/1/47 (H1N1)	0	2560	0	0	0	0	0	0	0	0	0	0	0	0
(3)A/Москва/0687/77	0	2560	0	0	0	0	0	0	0	0	0	0	0	0
(3)A/Москва/0778/77	0	2560	0	0	0	0	0	0	0	0	0	0	0	0
(3)A/Москва/0782/77	0	2560	0	0	0	0	0	0	0	0	0	0	0	0
(4)A/Новосибирск/02159/77	0	2560	0	0	0	0	0	0	0	0	0	0	0	0
(4)A/Новосибирск/02273/77	0	1280	0	0	0	0	0	0	0	0	0	0	0	0
(5)A/Виктория/35/72 (H3N2)	0	0	0	5 120	0	160	0	0	0	0	0	0	640	0
(6)Гомологичный	—	—	5120	40 960	5120	5120	5120	5120	5120	5120	5120	5120	5120	5120

Table 2. Interaction of Newly Isolated Strains With Antisera to Purified Hemagglutinin of Different Subtypes

Note: Reciprocal HIT titers are given

Key:

1. Antigens
2. Sera
3. Moscow
4. Novosibirsk
5. Victoria
6. Homologous

(1) Антигены	(2) Сыворотки				
	(3) А/лош/Пр/1/56—Гонконг/1/68 (270)	(4) А/лош/Пр/1/56—Виктория/3/75 (540)	(5) А/лош/Пр/1/56—PR8/34 (270)	(6) А/лош/Пр/1/56—FM/1/47 (540)	(7) А/лош/Пр/1/56—Нью-Джерси/8/76 (270)
(8) А/Москва/0687/77	0	1/18	1/3	1/6	1/3
(8) А/Москва/0675/77	0	1/18	1/3	1/17	1/3
(9) А/Хабаровск/034561/77	0	1/18	1/9	1/2	1
(10) А/Новосибирск/02279/77	0	1/18	1	1/6	1/3
(11) А/Полтава/0209/77	0	1/18	1/3	1/2	1
(9) А/Хабаровск/0787/77	0	1/9	1/9	1/6	1/9
(9) А/Хабаровск/0654/77	0	1/9	1/4	1/6	0
(8) А/Москва/0897/77	0	0	1/3	1/6	1/9
(11) А/Полтава/0275/77	0	0	1/9	1/2	1/9
(12) А/Владивосток/0368/77	0	0	1/9	1/6	1/9
(9) А/Хабаровск/0724/77	0	0	1/9	1/6	1/9
(12) А/Владивосток/0423/77	0	0	0	1/6	1/4
(12) А/Владивосток/0374/77	0	0	0	1/6	1/9
(9) А/Хабаровск/0534/77	0	0	0	1/6	1/9
(11) А/Полтава/0274/77	0	0	0	1/6	1
(11) А/Полтава/0237/77	0	0	0	1/9	1
(11) А/Полтава/0234/77	0	0	0	1/6	1
(13) А/Белгород/0236/77	0	0	0	1/18	1/3
(13) А/Белгород/0231/77	0	0	0	1/6	1
(14) А/Кемерово/0440/77	0	0	0	1/9	1
(9) А/Хабаровск/034574/77	0	0	0	1/6	0
(9) А/Хабаровск/034457/77	0	0	0	1/6	0
(13) А/Белгород/0232/77	0	0	0	0	1
А/PR8/34	0	0	1	1/9	0
А/FM/1/47	0	0	0	1	1/9
(15) А/Нью-Джерси/8/76	0	0	0	1/9	1
(16) А/Виктория/3/75	1/9	1	0	0	0

Table 3. Antigenic Specificity of Neuraminidase from New Strains Isolated in 1977: Reciprocal homologous titers are indicated in parentheses

Key:

1. Antigens
2. Sera
3. A/horse/Pr/1/56--Hong Kong /1/68
4. A/horse/Pr/1/56--Victoria/3/75
5. A/horse/Pr/1/56--PR8/34
6. A/horse/Pr/1/56--FM/1/47
7. A/horse/Pr/1/56--New Jersey/8/76
8. Moscow
9. Khabarovsk
10. Novosibirsk
11. Poltava
12. Vladivostok
13. Belgorod
14. Kemerovo
15. New Jersey
16. Victoria

(the heavy hemagglutinin chain), which is either not revealed or emerges above the upper edge of NP protein (*A*, *3-5*, gel *B*). Thus this polypeptide from epidemic strains is not identical in relation to migrational mobility in gel to HA_1 polypeptide in the virus FM/1/47.

Minor P polypeptides and neuranimidase were either not revealed or detected in quantities too negligible to permit a conclusion concerning their similarities or differences.

Discussion

In the 44 years since the time the viral etiology of human influenza was established, the antigenic profile of the strains changed in the following order: Strain A(HONl) dominated from 1933 to 1946, A(HlNl) dominated from 1947 to 1956, A(H2N2) dominated from 1957 to 1967, and A(H3N2) dominated from 1968 to 1977. Inasmuch as the last three strain changes occurred with a ten year interval, virologists expected occurrence of a new variant in 1978.

However, the first outbreaks in November 1977 were elicited by type A influenza virus strain revealing no antigenic relationship to the variants A/Victoria/3/75, A/Victoria/112/76, and A/Texas/1/77. Having studied the new strains, we concluded that they were related to the previously known type Al influenza virus (based on the old classification), or virus A(HlNl), as designated by the WHO classification adopted in 1971 (6). A relationship of strains that caused an influenza outbreak in November-December 1977 to virus A(HlNl) was established by a complex of diagnostic tests. In this case what was important was the fact that antisera, prepared by different institutions using different animals, to viruses containing hemagglutinin Hl inhibited the new strains to one-fourth or the full homologous titer. It should also be emphasized that a relationship of the neuraminidase of new strains to the Nl subtype was established with the use of antisera to recombinants, which precluded the effect of steric inhibition.

Type A(HlNl) viruses are nonhomogeneous (7-9). The strains that appeared in 1947-1949 differed from strains isolated in recent years of circulation of this virus type.

Data presented in this communication persuasively demonstrate that the newly isolated strains are related to type A(HlNl); however, there are not enough data to answer the question as to the degree to which they are related to strains that had been circulating in 1947-1957.

Even though regular change of the antigenic profile of epidemically active type A strains was firmly established as a fact, the question as to whether types of viruses that had once lost their epidemic activity could return

remained unclear. This communication provides the first evidence indicating that type A(H1N1) influenza viruses that had been circulating in 1947-1957 reacquired epidemic activity and caused the fall 1977 epidemic outbreak in the USSR.

The authors extend their deep gratefulness to Dr R. Webster (St. Judas Hospital, USA) for providing specific antisera to the different subtypes of hemagglutinin.

We express our gratefulness to colleagues of the laboratory for diagnosis of viral infections and clinical immunology of the Institute of Virology imeni D. I. Ivanovskiy and of the Moscow, Khabarovsk, Vladivostok, Novosibirsk, and Poltava research bases for their operational information and for their prompt submission of influenza data to the regional center.

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COMPARATIVE STUDY OF THE BIOPHYSICAL PROPERTIES OF TYPE A INFLUENZA VIRUS RIBONUCLEOPROTEINS

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978, pp 142-146

[Article by A. K. Gitel'man, V. T. Ivanova, L. Ya. Zakstel'skaya, and A. G. Bukrinskaya, Institute Of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow]

[Text] The sedimentation and density properties of ribonucleoproteins were studied with different strains of type A influenza virus: A/turkey/Wisconsin/66, A/swine/Iowa/15/31, A/horse/Prague/56, antigenic A (H3Neq1), and biological (MRC-11) recombinants of the A/Port-Chalmers/1/73 strain. The ribonucleoprotein (RNP) of all viruses studied was heterogeneous, with sedimentation occurring in the 30-70 S zone. Variability was revealed in the distribution of fragments in different strains. The highest radioactivity was found in the zone of "heavy" fragments (60-70 S) for the A/turkey/Wisconsin/66 and MPC-11 strains, while for the A/horse/Prague/56 and A/swine/Iowa/15/31 strains radioactivity dominated in the 40-50 S zone. The distribution of radioactivity varied in different experiments for the recombinant A(H3Neq1). The buoyant density of all components in cesium chloride was found to be similar, 1.34-1.36 gm/cm^3

Influenza virus occupies a special place in the group of viruses with a negative genome in connection with a number of unique features in its structure and reproduction. We know that the genome of type A influenza virus consists of eight separate fragments (1,2). The nucleocapsid of these viruses is also fragmented, and its fragments undergo sedimentation in sucrose concentration gradients in the 30-75 S zone (3,4).

Our previous research showed that the sedimentation properties of ribonucleoproteins (RNP) vary somewhat in different strains. Thus in distinction from RNP isolated from avian viruses (5), a component with a

sedimentation coefficient of 60 S dominated in RNP from A2/Hong Kong/1/68, a strain of human origin.

We were interested in studying a broader spectrum of viral strains and revealing a relationship between origin and the sedimentation distribution of nucleocapsid fragments. For this purpose we selected strains isolated from birds and mammals, an epidemic strain, and an antigenic recombinant of human and animal viruses.

Materials and Methods

The following viruses were used: A/turkey/Wisconsin/66 (Hav6N2), A/swine/Iowa/15/31 (HswN1), A/horse/Prague/56 (HeqlNeq1), the antigenic recombinant (H3Neq1), *consisting of hemagglutinin from the strain A/Port-Chalmers/1/73 (H3) and neuraminidase from the strain A/horse/Prague/56 (Neq1), and the biological recombinant MRC-11**having the antigenic structure of A/Port-Chalmers/1/73 (H3N2) and the biological properties of A/Puerto Rico/8/34.

Prior to the work, all strains underwent two passages at maximum dilutions in chick embryos. Labeled virus preparations were obtained by adding 200 millicuries/(Ki) of ^{3}H-uridine (specific activity 22 curies/millimole) into the infected embryo. Concentrated and purified virus preparations were obtained in accordance with a method described earlier (6). RNP was obtained by treating purified virions with Twin-20 nonionic detergent in alkaline conditions in accordance with Hosaka's method (7). The disintegrated virus preparations were applied to a 16-30 percent linear sucrose gradient made with 0.01 M tris-HCl, pH 7.4, as the buffer and containing 1 mM EDTA, and they were centrifuged in a Bekman L5-50 ultracentrifuge with an SW27.1 rotor for 15-17 hours at 22,000-24,000 rpm at 4°C.

Large and small 50 and 30 S ribosomal subunits in TKE buffer (0.01 M, pH 7.4 tris-HCl; 0.025 M KCl; 0.001 M EDTA) were used as labels. Density analysis was performed in a cesium chloride gradient (6).

Results

Figure 1 shows the radioactivity distribution of RNP obtained from different influenza virus strains following centrifugation in a sucrose concentration gradient. Three experiments were performed with each strain. The results of these experiments were found to be similar.

As we can see from Figure 1*a*, the RNP of A/swine/Iowa/15/31 occupied a heterogeneous zone from 35-60 S, with radioactivity dominating in a zone near 50 S. The RNP of A/turkey/Wisconsin/66 (Figure 1*b*) distributed

*obtained from Dr Webster
**obtained from Dr Schild

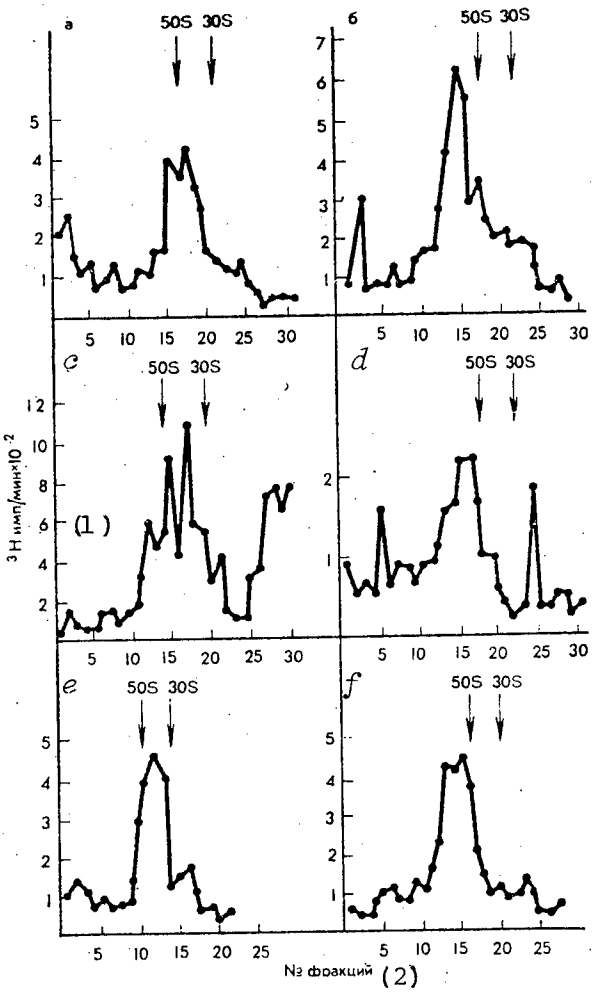

Figure 1. Sedimentation Characteristics of RNP from Influenza Virus Strains A/Swine/Iowa/15/31 (*a*), A/Turkey/Wisconsin/66 (*b*), A/Horse/Prague/56 (*c*), MRC-11 (*d*), A(H3Neq) (*e*,*f*): Disintegrated virus preparations were applied to a 15-30 percent linear sucrose gradient made with pH 7.4 0.01 M tris-HCl buffer containing 0.01 M EDTA, and centrifuged in an SW27.1 rotor for 16-17 hours at 22,000 rpm (*a*,*b*, *d*, *f*) and for 15 hours at 24,000 rpm (*c*, *b*).

Key:
1. ^3H, pulses/min·10^{-2}
2. Fraction number

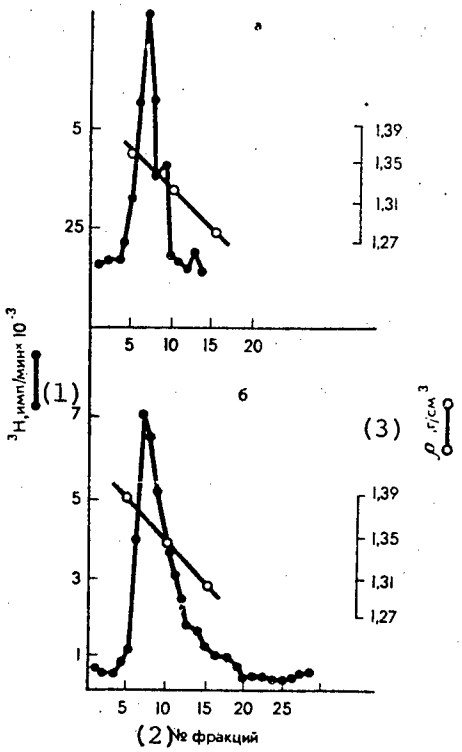

Figure 2. Analysis of Viral RNP in a Cesium Chloride Density Gradient: *a*--Sucrose Gradient Fractions No 12 (see Figure 1*e*) Were Collected, Fixed with 4 percent Formaldehyde, and Dialysed Opposite 0.01 M tris-HCl, 0.0011 M EDTA, pH 7.4 Buffer with 4 percent Formaldehyde; Then They Were Analysed in a Linear Cesium Chloride Gradient (1.18-1.43 gm/ml) in an SW 50.1 Rotor for 16 Hours at 36,000 rpm at 4°C; A/Horse/Prague/56 Strain; *b*--Radioactivity Distribution, Following Centrifugation (in the same conditions), of a Total RNP Preparation Isolated from the Biological Recombinant MRC-11.

Key:

1. ^{3}H, pulses/min•10^{-2}
2. Fraction number
3. gm/cm^3

itself in a heterogeneous zone with sedimentation coefficients from 30 to 70 S, the 65 S component dominating. RNP from A/horse/Prague/56 (Figure 1*c*) resolved into three components with sedimentation coefficients of 60, 50, and 40 S, exhibiting an identical quantity of radioactivity. RNP from the biological recombinant MRC-11 (Figure 1*d*) was distributed heterogeneously in a zone with sedimentation coefficients from 35 to 70 S, radioactivity dominating in the 60 S zone. Unambiguous results could not be obtained for RNP isolated from the antigenic recombinant A (H3 Neq 1). Radioactivity was distributed in a heterogeneous zone with the 50 S component dominating in some cases, such as for RNP from A/horse/Prague/56, and with the 70 S component dominating in other cases, as with RNP from the biological recombinant MRC-11 (Figure 1*e*, *f*).

RNP from these strains was subjected to density analysis.

Figure 2*a* shows radioactivity distribution, following recentrifugation in a cesium chloride gradient, of RNP components of A/horse/Prague/56 in the 40-60 S zone (gradient fractions No 12-18, shown in Figure 1*c*), while Figure 2*b* shows radioactivity distribution, following centrifugation, of a total RNP preparation isolated from the biological recombinant MRC-11. As we can see, in both cases radioactivity is exhibited in the same zone of a gradient having a density of 1.34-1.36 gm/cm^3.

The same results were obtained on analyzing RNP isolated from other strains. Thus density analysis of RNP isolated from different strains showed that all RNP components had a similar buoyant density.

Discussion

The goal of this work was to study the density and sedimentation properties of RNP from type A influenza strains of different origins. The research showed that RNP isolated from different strains has identical buoyant density (1.35-1.36 gm/cm^3), which indicates that the quantity of protein subunits associated with the viral genome is identical.

Analysis of the sedimentation properties of RNP isolated from different strains showed that RNP distributes itself in a heterogeneous zone within about 30-75 S. However, some differences were discovered in the sedimentation properties of RNP from different strains.

RNP from A/horse/Prague/56 and A/swine/Iowa/15/31 was dominated by components with lower sedimentation coefficients in comparison with RNP isolated from viruses of human origin (MRC-11).

Homogeneous results could not be obtained for the antigenic recombinant A(H3Neq1), the parent strains of which included human A/Port-Chalmers/1/73 virus and animal A/horse/Prague/56 virus.

A component with a sedimentation coefficient of 50 S dominated in some experiments, while the 70 S component dominated in others.

The differences in RNP sedimentation characteristics can be explained in two ways: 1) despite compliance with the conditions for obtaining highly infectious progeny, presence of incomplete forms in a population of different virus strains cannot be excluded. As we know, incomplete influenza virus forms contain a lower quantity of heavy RNA fragments (8), and owing to this they should contain a lower quantity of RNP; 2) the obtained differences could also be explained by presence, in different strains, of different sizes of genome fragments and, correspondingly, RNP fragments. In this case we would expect that the molecular weights of polypeptides from different strains would also vary. In fact, we know (9) that the molecular weight of hemagglutinin and neuraminidase differs for different strains. The strains used in our experiments also differed in relation to molecular weight of hemagglutinin Thus the molecular weight of hemagglutinin in MRC-11 was $7 \cdot 10^3$ daltons while that of other strains was $74 \cdot 10^3$ daltons. The molecular weight of nucleocapsid protein was found to be the same for all strains--$58 \cdot 10^3$ daltons. A detailed description of these results will be provided in a subsequent communication.

Thus, we can hypothesize that a direct correlation exists between RNP fragments undergoing sedimentation more slowly and the lower molecular weight of virion proteins.

To confirm this hypothesis, we would have to subdivide the different RNP fragments and identify the genes they contain.

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MATURATION OF INFLUENZA VIRUSES: FACTORS LEADING TO LOSS OF INFECTIOUSNESS

Moscow VOPROSY VIRUSOLOGII in Russian No 2, 1978 pp 137-142

[Article by A. G. Bukrinskaya, Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow]

[Text] Published data and data from the author's research on factors acting upon maturation of influenza virus and causing loss of infectiousness of viral progeny are presented. One of these factors is the inability of the cell's proteolytic enzymes to break down hemagglutinin. This phenomenon was discribed by German and American researchers in 1975. Another factor is decrease in content or absence of viral matrix protein in cell membranes. An abortive reproduction cycle is encountered in both cases. The hypothesis is suggested that regulation of maturation is achieved through coiling of nucleocapsids into helices upon their interaction with membrane protein.

Today's virology is characterized by the fact that molecular biology, which had always made extensive use of virological models, is now generously beginning to pay its debts. We now know a great deal about what the infectiousness of viral progeny depends on. Without a doubt we will soon add greater clarity to trivial concepts in "classical" virology such as "pathogenicity," "virulence," and "toxicity." Much is now known about the stages of intracellular reproduction of viruses. We have come to understand the mechanisms of adsorption and penetration of viruses into cells, the mechanisms of transcription and translation of "minus"-stranded viruses (nothing can be said for the moment about replication), and the role of viral nucleocapsids, which not only transport viral polymerase into the cell and promote its contact with the viral matrix, but also take a part in both regulation of viral reproduction and, apparently, switching from transcription to replication. The veil over the mysterious mechanism of discrimination between cellular and viral matrixes in infected cells and over the key role played by initiation factors in initiation complexes is being raised.

Influenza virus occupies a special place among viruses with a negative genome.

Everything said above about these viruses can be attributed to influenza virus only with a certain qualification, in connection with some unique properties of its reproduction. Much is known about its penetration into the cells, but we are not sure whether nucleocapsid fragments disperse or somehow maintain their initial arrangement in the cytoplasm. Transcription proceeds within the nucleocapsid in the universal way, but it is preceded by a mysterious phase of interaction between viral RNA and the nucleus, producing the possibility for transcription and the entire subsequent course of reproduction. A switch is made from transcription to replication through a mysterious pathway and in an unknown part of the cell, and some of the daughter nucleocapsids find one another in the complex pool of cellular ribonucleoproteins, arranging themselves correctly and forming a daughter genome that includes itself within the composition of viral particles. These problems have special significance in connection with the fact that intracellular reproduction of viruses can go a long way in explaining the unique capability viruses have for recombinations leading to antigenic variability, which predetermines the unusual epidemiology of influenza.

One of the least studied stages of reproduction is assembly or maturation of viral particles. We still are unsure how nucleocapsids disengage themselves from intracellular synthesis and acquire the capability for approaching the membrane, aligning themselves beneath it, and then including themselves into the composition of a budding viral particle. And yet it is precisely in the concluding stages of virus formation that we discover the factors limiting infection and, in a number of cases, defining the quantity of virus produced, its infectious activity and, thus, the system's permissiveness and the type of infection--productive, abortive, or chronic.

As a virus matures, three viral polypeptides interact with cellular lipids: Two glycoproteins (hemagglutinin and neuraminidase) and proteins of the inner membrane--matrix proteins (M-proteins). Glycoproteins stabilize on the outer surface of cell membranes, penetrating a certain depth into the lipid layer. Judging from the fact that complete removal of glycoproteins from the virion surface with proteases does not noticeably change the properties of the lipid bilayer, glycoproteins do not penetrate very deeply. These proteins are synthesized in rough membranes and are transported to plasma membranes through smooth membranes. Post-translational modification of polypeptides--glycosylation and splitting--occurs during transportation in smooth and, in part, in plasma membranes. Glycosylation is a stepped process in which carbohydrate molecules are gradualy suspended from a chain attached to a strictly defined portion of the polypeptide molecule (it is possible that the place of attachment of sugars predefines some biological properties of hemagglutinin, particularly its antigenic structure). A polypeptide is split in a strictly predefined section as well, where a sequence of seven amino acids, the so-called palinder (1), identical in all type A influenza viruses, is located. This sequence of amino acids is recognized by cellular proteases, and as a result the hemagglutinin molecule is split into two subunits--a heavy and a light one, joined together by a few sulfhydryl bonds. Each of them contains its own antigenic determinants, and the molecule's hydrophobic end locates itself on the light subunit. It would be important

to note that the unsplit precursor also has hemagglutinizing activity. The point of view based on data of Lazarowitz et al.(2) that splitting does not influence the infectiousness of viral progeny had existed for a number of years. But an article by Klenk et al.(3) appearing in 1975 and an article by Lazarowitz and Choppin(4) in the same issue of that journal refuted this idea and clearly demonstrated that splitting significantly heightens the infectiousness of the virus. These data indicate that in addition to its role in adsorption, hemagglutinin has another as yet unknown function associated with a virus's capability for infecting a cell.

This property of hemagglutinin was extrapolated to the mechanism of abortive infections. The hypothesis was suggested that low infectious activity of progeny in a number of abortive infections stems from absence of the appropriate proteases in the cells splitting the initial polypeptide target of hemagglutinin. These ideas were placed at the basis of a conception suggested by Choppin(5) in which he attempted to explain the mechanism of not only abortive but also persistent infections by the absence of appropriate proteases in nonpermissive systems. The weak side of this conception is that absence of splitting may be explained not by absence of the appropriate proteases but rather by unusual conformation of the polypeptide precursor, as had been demonstrated with paramyxoviruses as the model(6). We would more likely expect that production of viral particles should be blocked at the level of virion assembly, and that this should depend primarily on defects in synthesis, structure, or position of the membrane, matrix protein(M protein) built into the lipid bilayer on the inner side. In the virion, this protein forms a continuous molecular layer beneath the lipid membrane, imparting stability to the latter. M-protein contains up to 75 percent neutral amino acids, and it is this unusual chemical composition that makes it highly hydrophobic and lipophilic--that is, capable of close interaction with the lipid bilayer and capable of intermolecular bonding and protein-protein interactions(7,8). The role of M-protein as a mediator in cementing viral particles is the product of these unique properties; on one hand it binds with lipid and glycoproteins incorporated within them, and on the other hand it is specifically "recognized" by nucleocapsids accumulating in the cytoplasm. Thus M-protein may play a key role in assembly of viral particles, and its inclusion into plasma membranes may be precisely the concluding stage, immediately followed by linkage of nucleocapsids to the appropriate portions of the membrane and budding of the viral particles. It follows logically from the above that M-protein can be a limiting factor in viral maturation, predetermining correct assembly of the viral particles, the time of virus production, and the size of the yield--that is, events responsible for the productive and abortive types of infections. Our hypothesis explaining the nonpermissiveness of some cell systems in relation myxoviruses and paramyxoviruses is based on these properties of M-proteins. The experimental facts at the basis of the hypothesis are as follows.

When Erlich's ascitic carcinoma cells were infected with influenza virus (classical avian plague), in the late stages following infection the cells produced scanty viral particles distinguished from the standard

virus in relation to a number of properties. They were noninfectious (9,10) and they had unusually brittle capsids (11). When purified in sucrose and cesuim chloride density gradients, the ascitic virus disintegrated down to subviral particles--nuclides ("cows") devoid of a significant proportion of lipids and glycoproteins. These particles were devoid of hemagglutinating and neuraminidase activity, and electron microscopy revealed extensive defects in the layer of surface bulges ("commissures"). Fixation of the viral particles with formaldehyde stabilized the viral capsid and prevented further loss of lipids and glycoproteins upon recentrifugation in density gradients.

Electrophoretic analysis of the polypeptides of the ascitic particles in polyacrilamide gels revealed the same spectrum of structural viral proteins as in standard allantoic virus, though the ratio of these proteins differed. Matrix protein, the quantity of which was significantly lower as compared to nucleocapsid protein, experienced the most dramatic changes, and in a number of viral preparations this protein could not be detected in its usual location (11). Reduction of the quantity of matrix protein in ascitic virions agrees well with the unusual brittleness of these particles, and it is apparently the reason for instability of the lipid bilayer and loss of glycoproteins. These data on the role of M-protein in stabilization of the viral capsid were subsequently confirmed by Kendal et al. (12).

There may be several reasons for the reduced quantity of matrix protein in ascitic virus: 1) limited expression of its gene occurs in cells; 2) protein synthesis occurs, but the protein is built into the lipid bilayer incorrectly, and it cannot enter into the composition of virions; 3) the protein is included in ascitic virions, but due to unusual associations with lipids in the ascitic cells it is easily lost during purification of the virus; 4) the usual quantity of protein is in the composition of virions, but it exists in a different form, aggregated for example, and it cannot be identified in polyacrilamide gels. Research going on today will permit us to clarify which of the reasons holds, though obviously the last two are the least probable.

The obtained data permit the suggestion that disturbance of synthesis or introduction of matrix protein into the membrane causes disorganization of virus assembly, and that it is a direct cause of abortive reproduction of influenza virus.

It appears that defects in synthesis or the location of matrix protein are typical of not only the nonpermissive system we described. Skehel also revealed a dramatic decline in synthesis of matrix protein in L-cells abortively infected by influenza virus, and he discovered that mRNA is not produced for matrix protein in the system. Skehel suggests that translation of influenza virus protein occurs nonsimultaneously, that M-protein is among the "late" proteins (13,14), being a product of secondary transcription, and that it is not produced during primary transcription.

However, tests (15,16) showed that all viral proteins, including M-protein, are products of primary transcription of the viral genome.

As was noted above M-protein is specifically "recognized" by nucleocapsids, after which budding of the viral particle occurs quickly. However, we still do not know how nucleocapsids approaching cell membranes interact with M-protein incorporated within them, and we do not know whether or not the structure and conformation of nucleocapsids change in this connection. Analysis of nucleocapsids in our nonpermissive system, characterized by dramatic decline in the content of M-protein in cell mambranes, is interesting from this point of view. Erlich's ascitic cancer cells infected by paramyxovirus (Sendai virus) were found to be such a system. In this case the cells did not produce virions (17), though particles with a density intermediate between a virus and a nucleocapsid were discovered in cytoplasm and in culture fluid; it was found by electron microscopy that these particles were nucleocapsids associated with membranes. These particles were found to contain nucleocapsid proteins (P and NP) and both viral glycoproteins and their precursors (NHo, Fo, and F)--in other words all viral proteins with the exception of M-protein. In both systems of infection by influenza and Sendai viruses, the nucleocapsids isolated from cytoplasm were uncoiled strands with a diameter of 3-6 nanometers, while nucleocapsids isolated from the cytoplasm of permissive cells were rigidly coiled and had the same diameter as that of nucleocapsids isolated from virions (13-15 and 15-17 nanometers for influenza and Sendai viruses respectively). In both systems the nucleocapsids underwent sedimentation more slowly, and they had a greater density than nucleocapsids isolated from permissive cells. Perhaps the latter situation is associated not only with change in conformation but also absence of some protein subunits. In fact, analysis of the proteins with dodecyl sodium sulfate and polyacrilamide gel electrophoresis demonstrated a decline in the relative concentration of the major nucleocapsid protein, NP, in relation to the minor proteins, P.

Thus in cells having a reduced quantity of M-protein or in cells in which M-protein is absent from cell membranes, cytoplasmic nucleocapsids are not coiled, and infectious viral particles are not produced.

It should be noted that an insignificant fraction of coiled nucleocapsids characterized by normal buoyant density was detected in Erlich's cells infected by influenza virus, and it was precisely these nucleocapsids that were isolated from virions produced by cells in meager amounts. This permits the hypothesis that only coiled nucleocapsids can become encapsulated and be included in the composition of virions.

The hypothesis that interaction of M-protein with nucleocapsids, which by itself is the product of the hydrophobic properties of M-protein and its capability for protein-protein interactions, leads to change in conformation of nucleocapsids and their coiling follows from the discussion above. Acquisition of such conformation is a prerequisite for inclusion

of the nucleocapsid into the composition of virions. In this case M-protein should be given a regulatory role in selection of the fraction of nucleocapsids, to be included in the virion, from the pool of uncoiled (or partially coiled) nucleocapsids taking part in transcription and replication. Were this hypothesis to be confirmed, we would find the missing link explaining the mechanism of the subsequent stages in maturation of lipid-containing "minus"-stranded viruses.

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DATA FOR DETERMINATION OF MAXIMUM PERMISSIBLE CONCENTRATION OF DIAMINODIPHENYLSULFONE IN THE ATMOSPHERIC AIR OF POPULATED REGIONS

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 3-5

[Article by L. Kh. Tsyganovskaya, candidate of medical sciences; L. I. Volokhova, candidate of chemical sciences; and V. M. Voronin, candidate of medical sciences, Institute of General and Municipal Hygiene imeni A. N. Sysin, USSR Academy of Medical Sciences, Moscow, submitted 16 Jun 77]

[Text] 4,4'-Diaminodiphenylsulfone (DDS) is a promising product for the synthesis of highly thermostable synthetic fibers for special purposes. DDS can penetrate into atmospheric air in the form of aerosol in the technological process of manufacturing sulfone-T fiber.

According to the results of the studies of N. G. Ivanov and L. V. Mel'nikova, the LD_{50} constitutes 1400 mg/kg (1624-1207), Lim_{ac} is 34 mg/m^3 and Lim_{ch} is 9 mg/m^3 for albino rats. The substance has marked cumulative properties, and the coefficient of accumulation is 0.7; it is in hazard class III. The MPC [maximum permissible concentration] has been set at 5 mg/m^3 in the air of industrial buildings.

DDS is an effective agent in the treatment of leprosy (Brown, 1969). The maximum tolerated daily dosage is 200 mg; higher doses induce anemia and methemoglobinemia (V. K. Loginov et al.; Shepard). It has been demonstrated that sulfones affect detoxifying function of the liver (G. A. Chuchelin and Z. F. Kadantseva) and transamination processes in the liver (I. M. Khalimanchuk).

According to data in the literature, cases of sensitization have been observed with long-term intake of DDS as a drug (Brown, 1963; Shepard; Verma et al.).

In our experiments, we studied the changes in the animal organism after intake of DDS in the form of aerosol inhalation. The direction of effects of this agent was determined in preliminary 2-week experiments, involving around the clock exposure to DDS aerosol in a concentration close to the MPC for air of industrial buildings. A minor increase in activity of alanine transaminase (SGPT) and marked decrease in activity of aspartate transaminase (SGOT)

in blood serum [2.61±0.3 mmole/(h•ℓ) in the experimental group and 1.58±0.19 mmole/(h•ℓ) in the control]. No elevation of blood methemoglobin level was demonstrable in experimental animals (1.6±0.5 versus 1.1±0.6 g% in the control). In the same experiment, we made an effort at determining the mechanism of DDS metabolism in the organism. We found increased induction of cytochrome r-450 in the liver (1.07 in the experimental group versus 0.62 in the control); this indicates that DDS is metabolized in the liver with the help of enzymes located in the endoplasmic reticulum (microsomal fraction) of hepatic cells.

In order to investigate the nature of changes in the organism in the case of prolonged intake of low concentrations of DDS, 60 male albino rats, weighing 180-200 g, were submitted to around the clock inhalation for 3 months. The animals were distributed in 4 groups: the 1st was exposed to DDS aerosol in a concentration of 0.5±0.2 mg/m^3; the 2d, in a concentration of 0.13±0.1 mg/m^3; the third, in a concentration of 0.05±0.03 mg/m^3, while the 4th served as a control. We assessed the toxic effects of low concentrations of DDS aerosol in chronic experiments on the basis of a set of integral parameters: we studied the dynamics of weight gain, functional state of the nervous system was evaluated by means of the threshold-summation index (TSI), functional state of the liver was determined on the basis of blood serum cholinesterase and transaminase activity; determination was made of immunological changes in the animal organism (Hoigne reaction, IHAR [inhibited hemagglutination reaction], as well as changes in morphological composition of blood. Upon terminating the experiment, we conducted a morphological examination of the lungs, liver and spleen (of 4 animals in each group). We tested the activity of succinate and lactate dehydrogenases, DPN diaphorases and cytochromoxidase in the liver and lungs.

Determination of the dynamics of weight gain revealed a significant lag of this parameter in the 1st group of animals throughout the experiment, probably as a result of the deleterious effect of the agent. In this group of animals, we observed elevation of TSI, starting in the 2d week of inhalation, and it became statistically reliable thereafter, in the 4th and 10th weeks. There was some decline of TSI in the 2d group of animals, which became reliable only by the 8th experimental week. TSI was on the control level in the animals of the 3d group.

Biochemical tests revealed a tendency toward diminished activity of blood cholinesterase in the 1st and 2d groups, and it became statistically reliable in the former by the 10th day of inhalation, persisting to the end of the experiment. These indices were virtually the same as in the control in the 3d group of animals.

There was a change in blood serum transaminase activity. In the 1st group of animals, we observed a statistically reliable increase in activity of both SGPT and SGOT throughout the experiment; in the 2d group, there was a reliable increase in activity of the latter. In the 3d group of animals, activity of both transaminases differed little from the control.

In view of the fact that there are indications in the literature about sensitizing properties of DDS, we evaluated immunological changes in animals chronically exposed to DDS. Analysis of precipitating (Hoigne reaction) and agglutinating (IHAR) antibodies in blood serum after inhalation for 3 months and after 2 weeks of the recovery period failed to demonstrate substantial differences from the control.

A study of dynamics of blood morphology revealed some increase in percentage of eosinophils in the blood of the 1st group of animals, which started in the 2d month and increased in the 3d (4.0±0.32 and 6.2±0.8, respectively, versus 1.4±0.24 in the control). Some increase in eosinophils was found in the 2d group of animals, in the 3d experimental month (4.0±0.74%). Morphology of the blood of the 3d group of animals differed little from the control.

Histological examination of the lungs revealed the most marked, chronic interstitial changes in the 1st group of animals, who presented thickening of interalveolar septa due to proliferation of cells, areas of acute inflammation (peribronchitis) and emphysema. Somewhat less marked disturbances were demonstrated in the 2d group of animals. The nature and severity of changes in the lungs of the 3d group of animals were similar to those in the control.

Swelling of Kupffer cells in some areas of the liver, erosion of margins and structure of the cytoplasm of some hepatocytes, which could be evaluated as a sign of dystrophic changes. Histochemical studies of redox enzyme activity revealed that, with increase in DDS concentration, there was an increase in succinate dehydrogenase activity, and this process was noted in both the organ of direct contact, the lung, and the liver (mainly in the central lobes).

As a result of the experiment, it was established that inhalation of DDS has a general toxic effect, which is manifested by impaired weight gain and persistent change in functional state of the nervous system. However, the liver is apparently the organ that is chiefly involved, which is consistent with the data in the literature and the fact that we demonstrated metabolism thereof by means of microsomal enzymes of hepatic cells; and, while the changes in the liver were merely functional under the influence of DDS in a concentration of 0.1 mg/m^3 (2d group), pathomorphological changes were also observed under the influence of a concentration of 0.5 mg/m^3 (1st group). In the organ where there was direct contact with the agent under study, the lungs, we observed dual changes: hemodynamic disturbances and chronic inflammatory signs. In the 2d group of animals, and especially the 1st, chronic changes in interstitial tissue were prominent. We cannot rule out the factor of chronic intoxication of the organism from the mechanism of development of the last-mentioned changes.

All of the manifestations of general toxic effect of DDS were the most marked in animals exposed to a concentration of 0.5 mg/m^3; changes also occurred under the influence of 0.1 mg/m^3; only 0.05 mg/m^3 was found to be ineffective,

according to all of the selected parameters. It is recommended as the mean daily MPC for atmospheric air, and it was approved by the section for sanitary protection of atmospheric air.

Conclusions

1. DDS aerosol in concentrations of 0.5 and 0.13 mg/m^3 has a deleterious effect on the animal organism. All changes are the most marked with exposure to this agent in a concentration of 0.5 mg/m^3.

2. When used in a concentration of 0.05 mg/m^3, DDS did not elicit reliable changes indicative of a deleterious effect on the organism in any of the chosen parameters. The mean daily MPC thereof for the atmospheric air of populated areas has been set at 0.05 mg/m^3.

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EXPERIMENTAL SUBSTANTIATION OF MAXIMUM PERMISSIBLE CONCENTRATION OF HEXYLURE IN WATER

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 15-18

[Article by S. I. Avdyushkina, All-Union Scientific Research Institute of Hygiene and Toxicology of Pesticides, Polymers and Plastics, Kiev, submitted 14 Jun 77]

[Text] The active principle of hexylure is 3-cyclohexyl-5,6-trimethylene uracil. The chemically pure product is a white, crystalline substance with a melting point of 315.6-316.8°C and molecular weight of 234.3. Solubility in water constitutes 6 mg/ℓ at 25°C. It is produced in the form of 80% wetting powder. Studies were conducted with the technical-grade product, and the concentrations and doses were calculated for the active principle.

The conventional methodological system was used as the basis of our studies. There was no change in color and transparency of water in model reservoirs after addition of hexylure. In order to determine whether this agent could affect organoleptic properties of water and to set threshold concentrations according to this parameter, we tested the effect of hexylure on the odor and flavor of water. Different concentrations (from 0.1 to 6.0 mg/ℓ) of this herbicide were prepared in dechlorinated tap water, and each dilution was tested three times. We determined the odor and flavor of the water by the method of closed team odorization, the results obtained were rated on a 5-point scale and submitted to statistical processing (G. N. Krasovskiy).

It was established that hexylure affects organoleptic properties of water, imparting a nonspecific flavor and odor to it. The threshold of perception of odor (intensity of 1 point) at 20°C was on the level of 1.95±0.76 mg/ℓ, and the practical threshold was at 4.14±0.9 mg/ℓ (rated at 2 points). The bottom confidence range of the threshold of odor perception constituted 0.4 mg/ℓ and that of the practical threshold, 2.3 mg/ℓ. Raising the water temperature to 60°C did not affect the threshold of odor perception and raised the practical threshold somewhat (1.3 mg/ℓ). There was a taste to the aqueous solutions of hexylure at the level of 2.61±0.91 mg/ℓ. The aftertaste was the limiting feature, with regard to effects on organoleptic properties of water: the bottom confidence range of the threshold of taste perception constituted 0.15 mg/ℓ and the practical threshold was 0.8 mg/ℓ.

We tested the stability of the product in aqueous solutions by demonstrating it at different times after addition to model reservoirs by the method of chromatography in a thin layer of aluminum oxide (V. I. Bobrova and L. S. Bernatskaya). The sensitivity of the method constitutes 0.025 mg/ℓ. Experiments were conducted with river water in open vessels, with base concentrations of 1.0 and 0.1 mg/ℓ. During the experiment, the water temperature fluctuated from 15 to 20°C, pH from 7.3 to 7.8. Hexylure was quite stable in water: 50% was destroyed in 50 days, total decomposition occurred in 5 months in the case of a concentration of 0.1 mg/ℓ and in 8 months, with a concentration of 1.0 mg/ℓ.

We determined the effect of hexylure on general sanitary conditions of reservoirs and processes of self-purification of water to remove organic pollutants by examining the dynamics of biochemical oxygen uptake (BOU) and nitrification processes. In concentrations of 10.0 and 1.0 mg/ℓ, hexylure stimulates both BOU_5 and BOU_{20}. Thus, with a concentration of 10 mg/ℓ, BOU_5 was 47% higher and BOU_{20} 34.5% higher than in the control. With hexylure in a concentration of 1 mg/ℓ, BOU_5 and BOU_{20} constituted 126.7 and 117.2%, respectively, as compared to control water. At these concentrations there was also negligible increase in production of ammonia nitrogen, nitrites and nitrates.

Concurrently with measurement of BOU and investigation of nitrification processes, we estimated dissolved oxygen, oxidizability, active water reaction and the effect of hexylure on development and extinction of saprophytic microflora. Hexylure did not affect these indices in all tested concentrations. With regard to effect on sanitary conditions of reservoirs, 1 mg/ℓ of this product is the threshold level, while 0.1 mg/ℓ is inactive.

We tested the toxicity of hexylure to warmblooded animals on white mice and rats. Since the mechanism of action of uracil derivatives has not been investigated, in selecting tests to evaluate toxicity of hexylure, we proceeded from its chemical structure and some of the data in the literature concerning the effects of this group of substances on the organism. We selected morphological composition of peripheral blood, functional state of the thyroid, activity of some redox enzymes (catalases, peroxidases), nucleic acid content of the animals' liver and pathomorphological changes in internal organs as the main parameters for evaluation of the toxic effect of hexylure. Morphology of blood, activity of catalase and peroxidase were studied by conventional methods; function of the thyroid was evaluated using radioactive iodine and thyroxine (V. K. Modestov et al.); we used the stand designed by I. I. Shvayko to immobilize the rats while measuring thyroid function. The method of V. V. Galkin and G. D. Berdyshev, in the modification of A. I. Silakova and S. N. Polishchuk, was used to assay nucleic acids of the liver.

Hexylure is a compound with low toxicity: when administered in the stomach in doses of 5 to 20 g/kg, there were no animal deaths. It has mild cumulative properties, and they are functional in nature. When administered in the stomach, hexylure lowered catalase activity and negligibly increased thyroid function for 4 months in experimental rats.

A chronic 10-month experiment on rats established that, in doses of 10 and 1 mg/kg, hexylure induces a reliable decrease in weight gain, percentile hemoglobin and erythrocyte count. A reliable depression of erythropoiesis was also noted in rats with a dosage of 0.1 mg/kg. There was also an increase in thyroid function under the influence of hexylure in doses of 10, 1 and 0.1 mg/kg. With all tested doses, the percentage of radioactive iodine uptake by the gland was higher in experimental rats than intact ones, and maximum uptake in experimental rats occurred after 12 h, the time being 1 day for control animals.

Studies of RNA and DNA content revealed that the maximum elevation of RNA level (by 23.8%) occurred after giving hexylure at the rate of 10 mg/kg; a dosage of 1 mg/kg increased RNA content of the rat liver by 21.9%, as compared to the control. There was no change in DNA content under the influence of the herbicide.

Since the lowest of the tested doses (0.1 mg/kg) elicited a reliable increase in thyroid function and changes referable to red blood cells in experimental animals, we conducted an additional 6-month experiment giving hexylure in a dosage of 0.01 mg/kg. In the second series of experiments, we determined only the indices that had changed reliably with a dosage of 0.1 mg/kg. It was established that the product does not affect the body of warmblooded animals in a dosage of 0.01 mg/kg.

Pathomorphological examination of animal organs* revealed that the main lesions develop in the thyroid, liver and brain as a result of hexylure poisoning due to doses of 10 and 1 mg/kg. Many regions of the thyroid presented significant proliferation of interalevolar and follicular epithelium; complexes of desquamated epithelial cells were encountered in the lumen of some follicles. We were impressed by the irregularity of dimensions of follicles; among them, we encountered large and cystically dilated ones. Some dissociation was found in the liver; there were parenchymal cells with polyploid nuclei, anuclear cells and small groups thereof. In the brain there was minor perivascular edema and in the cerebellum, elimination of some Purkinje cells. We observed vacuolization of the cytoplasm of some ganglionic cells and karyolysis; in some areas neurophagia was observed. We failed to detect pathomorphological changes in the viscera of experimental animals given 0.1 and 0.01 mg/kg hexylure.

Thus, chronic experiments established that 0.1 mg/kg is the threshold dosage of hexylure and 0.01 mg/kg is inactive.

Conclusions

1. The results of our studies enabled us to set the threshold concentrations of hexylure: 0.16 mg/ℓ for the organoleptic parameter, 1 mg/ℓ for the general sanitary one and 0.2 mg/ℓ (0.1 mg/kg) for the sanitary toxicological.

*This study was pursued with the consultant assistance of G. A. Rodionov, doctor of medical sciences.

2. If we compare the obtained data, we shall see that the threshold concentrations for the organoleptic (aftertaste) and sanitary toxicological parameters are on the same level. If hexylure had had a specific taste, we could have recommended the organoleptic index as the limiting one for sanitary control. However, since it does not have such a taste, the sanitary toxicological index should be considered the limiting index of harmfulness, and 0.2 mg/ℓ* should be set as the maximum permissible concentration of hexylure in water.

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HYGIENIC EFFICACY OF ADDITIONAL OZONE TREATMENT OF LIQUID INDUSTRIAL WASTE OF COMPLEX CHEMICAL COMPOSITION AND TOXICOLOGICAL CHARACTERISTICS OF TRANSFORMATION PRODUCTS

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 22-25

[Article by A. A. Korolev, M. V. Bogdanov, I. I. Karavayev and L. V. Mamonova, First Moscow Medical Institute imeni I. M. Sechenov and All-Union Scientific Research Institute of the Ministry of Railroads, Moscow, submitted 20 May 77]

[Text] Ozone treatment is considered the most promising for additional treatment of liquid sewage (P. F. Kandzas and A. A. Mokina). However, no studies have been made of the hygienic aspects of efficacy of ozone treatment of liquid waste of complex chemical composition.

Studies were conducted with biologically treated liquid sewage submitted to additional ozone treatment on a LOG-15 laboratory unit and ozonization station of Kalikinskiy Tie [Sleeper] Impregnating Plant. In order to assess the efficacy of ozonization, we determined the change in concentrations of phenols and benz(a)pyrene, COU [chemical oxygen uptake], BOU [biological oxygen uptake] and level of ether-soluble substances, organoleptic indices of liquid waste, etc.

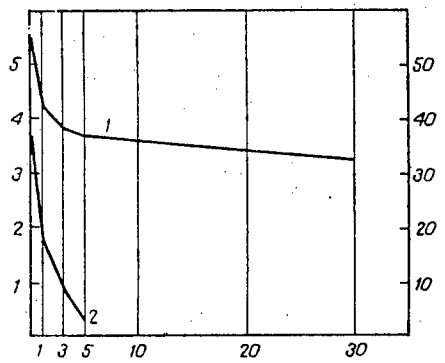
The results of our studies are submitted in Table 1, which shows that, after 20-30 min contact with ozone, liquid sewage was completely discolored and there was a significant decrease in organic substance content, as checked by the COU and BOU, amount of phenols and benz(a)pyrene. The concentration of the last two pollutants dropped to levels that are not uncommonly found in the water of superficial reservoirs (A. P. Il'inskiy et al.). At the same time, ozonized liquid waste still had a nonspecific odor graded at up to 4 points, which disappeared when diluted 3-5 fold. At the early stages of ozonization (up to 10 min), there was even some intensification of odor and change therein, apparently due to appearance in the waste of volatile products of transformation of organic substances, which could be aldehydes or carboxylic acids (A. A. Korolev).

A parallel study of changes in odor and COU while liquid waste stood in open vessels enabled us to gain some idea about the nature and stability of products of transformation of organic substances formed in the sewage after

ozonization. We found (see Figure) that, in the first 3 days after ozone treatment, the liquid waste lost its odor almost completely, while COU dropped by 30%, with little change thereafter, up to the 30th day. The observed correlation between dynamics of odor and COU serves as a confirmation of the hypothesis we previously expounded, as to the possibility of formation of unstable (due to volatility) products of transformation of organic substances of liquid waste. At the same time, the studies revealed that, after ozone treatment, a certain amount of odorless but highly stable products of transformation remains in the liquid waste, the chemistry and hazard of which are not known.

Table 1. Changes in some parameters of liquid waste after additional treatment with ozone

Index	Before ozone treatment	Time after ozonization, min		
		10	20	30
Transparency, cm	11	17	22	25
Dilution at which odor disappears	1:11	1:20	1:10	1:5
Dilution at which color disappears	1:50	1:3	0	0
pH	7,1	6,2	5,8	5,3
COU, mg/ℓ	250,0	80,5	64,0	40,0
BOU_5, mg/ℓ	10,5	—	—	2,7
Ether-soluble substances, mg/ℓ	180,0	53,5	40,3	29,3
Phenols, mg/	1,0	0,04	0,01	0,005
Benz(a)pyrene , μg/ℓ	50,0	—	—	0,02



Change in COU (1) and intensity of odor (2) of ozone-treated liquid waste. X-axis, time of observation (days); y-axis, odor (graded in points), on the left; COU (mg/ℓ), on the right.

Subacute experiments were conducted on albino rats to assess the extent and nature of possible toxic effect of products of transformation, formed with ozone treatment, on warm-blooded organisms. Three experimental groups of animals were given ozonized liquid waste, without and with 10- and 100-fold dilution thereof in tap water, for 1.5 months, from graduated water dishes. Control rats were given tap water. In view of the fact that the chemistry and action of products of transformation on the organism are not known, we

used a wide set of tests, which enabled us to investigate not only the general toxic, but possible specific (mutagenic, gonadotoxic, allergenic) effects of these products.

We observed the general condition, water intake, weight dynamics and morphology of the animals' blood. We determined the capacity of the central nervous system for summation of subliminal impulses. We determined blood and visceral cholinesterase activity, blood aminotransferase and peroxidase activity, as well as blood serum β-lipoprotein content. In order to assess the gonadotoxic effect, we made a complex study of functional state of spermatozoa and structural-functional elements of the testes. The mutagenic effect was evaluated by the method of anatelphase analysis of bone marrow cells. In order to determine whether there could be sensitization of the organism, we ran the reactions of erythrocyte and leukocyte lysis, precipitation in gel and agglomeration of leukocytes.

Table 2. Results of comparative study of the effects of products of transformation of biologically and ozone-treated liquid waste on albino rats in a chronic experiment

Indices	Products of liquid sewage transformation	
	biologically treated, 1:50 dilution	ozone treated, 1:10 dilution
Body weight	-	-
Fluid intake	-	-
Morphological composition of blood	+	-
Arterial pressure	+	-
Blood cholinesterase	+	-
Liver cholinesterase	-	-
Blood catalase	+	-
Blood peroxidase	+	-
Serum aminotransferases	-	-
Blood SH group content	+	-
Urea nitrogen in blood and urine	-	-
Urine creatinine	-	-
Conditioned reflexes	+	-
Threshold-summation index	+	-
Gonadotoxic effect	+	-
Mutagenic effect	-	-
Allergenic effect	-	-
Visceral weight coefficients	+	-
Pathomorphological examination of viscera	+	-

Note: + refers to reliable ($P<0.05$) changes and - to absence of changes.

The studies revealed that 2 out of the 20 tested parameters (brief elevation of cholinesterase activity and decline of blood peroxidase activity) changed in animals given products of transformation, which were contained in undiluted

ozonized waste. This warrants consideration of the effects of products of transformation as a low-intensity factor, with prevalence of processes of adaptation and compensation of body functions. In the subacute experiment, we also failed to demonstrate an effect on the reproductive function of the organism or allergenic effect of products of transformation. Reduction of concentration of the latter, due to 10-fold dilution of ozonized waste in water did not enable us to detect any influence whatsoever on the organism.

A chronic experiment was conducted on albino rats to obtain a definitive answer to the question of extent of danger and safety of products of transformation of ozonized liquid waste. The animals were given ozonized liquid waste diluted to 1:10, 1:50 and 1:250 to study the dose-effect function. In addition to the tests used in the subacute experiment, in the chronic one we determined several integral indices: conditioned reflex activity, blood SH group content, arterial pressure dynamics, renal function and morphological changes in organs. We investigated reproductive functions of experimental animals.

The results of the chronic experiment (Table 2) indicated that even in a 1:10 dilution, the products of transformation contained in ozone-treated liquid waste were unable to exert a deleterious effect on the organism, according to indices of both the general toxic and specific action. At the same time, the products of transformation, which remained in liquid sewage after biological treatment, in addition to manifestation of a general toxic action, induced disturbances referable to reproductive function and lost their toxic properties only when liquid waste was diluted to 1:250 (M. V. Bogdanov). Consequently, additional treatment with ozone of biologically treated liquid waste of a complex chemical composition makes it possible not only to reduce its toxicity sharply, but to obtain a water quality close to that of superficial reservoirs after ozonization, according to several indices. This makes it possible to dump such liquid waste into relatively small reservoirs with no restrictions on water consumption, while a mere 1:10 dilution of waste in the reservoir provides for a reliable enough level of transformation products, with respect to the sanitary and toxicological hazard index. Since some decontamination of liquid waste definitely occurs in the ozonization process, such waste could probably be used in some systems of industrial water supply, including tie-impregnating plants.

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TRANSFORMATION OF ORGANOPHOSPHORUS PESTICIDES, ANTHIO AND CHLOROPHOS, IN THE ENVIRONMENT

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 28-31

[Article by A. V. Bolotnyy, candidate of medical sciences; M. V. Pis'mennaya, candidate of chemical sciences, and S. L. Akoronko, All-Union Scientific Research Institute of Hygiene and Toxicology of Pesticides, Polymers and Plastics, Kiev, submitted 9 Sep 77]

[Text] According to data in the literature, organophosphorus pesticides (OPP), including anthio [0,0-dimethyl-S-(N-methyl-N-formylcarbamoylmethyl)-dithiophosphate] and chlorophos (0,0-dimethyl-(1-oxy-2,2,2,-trichloroethyl)-phosphonate] are readily susceptible to photochemical decomposition, with production of compounds that are more toxic than the original products. Rogor [0,0-dimethyl-S-(N-methylcarbamoylmethyl)-dithiophosphate] [phosphamide] is a product of anthio and it, in turn, is transformed into a P=0 analogue (L. G. Adeyshvili). The main product of chlorophos breakdown is 0,0-dimethyl-0,2,2-dichlorvinylphosphate--DDVP (G. Shrader; G. Mayer-Bode), which is then transformed into dichloracetaldehyde, dimethylphosphoric acid and other compounds (N. N. Mel'nikov). Like most OPP, chlorophos is stable in an acid medium, and it is rapidly hydrolyzed in an alkaline one. The rate of transformation of OPP increases significantly under the influence of ultraviolet radiation and high temperatures (M. A. Klisenko and M. V. Pis'mennaya).

We studied the level and conservation time of anthio and chlorophos, as well as their metabolites, rogor and DDVP in the environment, when these agents are used in orchard growing and viticulture. The orchard was sprayed with anthio using an OVS-A ventilator-tractor sprayer, at the rate of 1.2 kg pesticide per ha and 1000 ℓ working liquid. Vineyards were treated with chlorophos using an OMB-400 sprayer; normal outlay of the pesticide constitute 2 and 6 kg/ha, with 800 ℓ/ha working fluid. We assayed the pesticides and their metabolites in samples of leaves, apples, surface layer of soil (0-3 cm) and air over the treated areas using the method of thin-layer chromatography (L. G. Adeyshvili; M. A. Klisenko et al.). Sensitivity of demonstration of the agents in plants and soil constituted 0.1 mg/kg and in air, 1 μg per sample; accuracy of the method constituted 80-95%. Average samples were collected periodically, with three taken at each point.

Table 1. Dynamics of levels of anthio and its metabolite, rogor, in orchard objects (mg/kg)

When examined	Apples	Leaves	Branches	Soil under tree crowns	Inter-row soil
ANTHIO:					
Before treatment	n/d	n/d	n/d	n/d	n/d
After treatment, time:					
1 h	0.11	0.60	0.25	0.15	0.07
1 day	0.12	0.93	0.55	0.17	0.13
3 days	0.06	0.54	0.25	0.03	0.05
5 days	0.04	0.30	0.17	0.02	0.01
7 days	0.015	0.17	0.25	0.04	0.03
10 days	n/d	0.03	0.07	0.02	0.01
15 days	n/d	0.03	n/d	0.01	0.005
Harvest time (60 days)	n/d	n/d	n/d	n/d	n/d
ROGOR:					
Before treatment	n/d	n/d	n/d	n/d	n/d
After treatment, time:					
1 h	0.18	3.53	0.40	0.75	0.37
1 day	0.22	3.01	2.30	0.79	0.63
3 days	0.16	3.01	1.60	0.33	0.53
5 days	0.09	1.51	0.33	0.21	0.25
7 days	0.05	1.05	0.30	0.21	0.17
10 days	0.015	0.67	0.27	0.14	0.09
15 days	0.02	0.60	0.06	0.12	0.10
Harvest time (60 days)	n/d	n/d	n/d	n/d	n/d

Key: n/d--not demonstrated

As can be seen in Table 1, on the day of treatment, the anthio content of apples, leaves and soil did not exceed tenths of a milligram per kg; rogor was found in considerably larger quantities. The orchard objects could be put in the following order of decreasing contamination by anthio: leaves, branches, soil under the tree crown, apples and inter-row soil; for rogor, the following order was found: leaves, soil under crown, branches, inter-row soil and apples.

Anthio is retained for different periods of time in different media. Thus, no insecticide was found in apples 10 days after treatment or in branches 15 days after treatment, whereas the level thereof in leaves and superficial soil constituted hundredths or thousandths of a milligram per kg after 15 days. At this same period, residual amounts of rogor per kg leaves and superficial soil constituted tenths of a milligram, and hundredths of a milligram per kg apples. It may be assumed that retention time of anthio and rogor in orchard objects is determined by the initial level of the product in each of the media. No anthio or rogor was demonstrated in these objects at harvest time 2 months after treatment.

Table 2. Dynamics of levels of chlorophos and its metabolite, DDVP, in grape leaves, superficial soil (mg/kg) and air (mg/m^3) with the use of different dose rates

When examined	2 kg/ha						6 kg/ha					
	chlorophos			DDVP			chlorophos			DDVP		
	leaves	soil	air	leaves	soil	air	leaves	soil	air	leaves	soil	air
Before treatment	н/о	н/о	н/о	н/о	н/о	н/о	н/о	н/о	н/о	н/о	н/о	н/о
Day of treatment	17,20	0,24	traces	1,20	н/о	—	34,00	0,48	0,001	2,00	0,04	—
1 day later	8,80	0,49	0,0003	0,05	0,01	н/о	16,80	1,03	0,0004	0,90	0,05	—
3 days "	7,00	0,17	—	0,40	н/о	—	11,30	0,62	—	0,60	0,025	—
6 " "	1,16	0,09	н/о	0,30	0,007	0,0003	3,20	0,26	0,0003	0,50	н/о	0,001
10 " "	0,05	0,02	н/о	н/о	н/о	н/о	0,07	0,07	0,001	н/о	н/о	н/о
15 " "	0,05	0,002	—	н/о	н/о	—	0,03	0,02	—	н/о	н/о	—
30 " "	н/о	н/о	—	н/о	н/о	—	—	traces	—	traces	н/о	—

Key: н/о) not demonstrated -) not tested

The studies demonstrated secondary contamination by anthio and its metabolites of the air above the soil surface in the treated areas. However, the levels thereof in air were significantly lower than the MPC [maximum permissible concentrations] set for the air of work zones, and constituted ten-thousandths of a milligram per cubic meter. Typically enough, on the day of treatment there was only anthio in the air (0.0007 mg/m^3), and on subsequent days the concentration of rogor was higher in air than anthio. Anthio persisted in the air for 7 days and rogor, for 10 days.

The obtained data are indicative of intensive transformation of anthio into rogor in the environment. The level of the metabolite and retention time were significantly higher in orchard objects than those for anthio.

The distribution of chlorophos in the environment when used to treat vineyards depended on the dosage used. On the day of treatment at the rate of 6 kg/ha, the chlorophos content of grape leaves and superficial layer of soil was twice as high as with the use of 2 kg/ha (Table 2). At this same period, under similar meteorological conditions (air temperature 29°C, relative humidity 76-82%, air velocity 1.5-1.6 m/s), chlorophos was demonstrable in the air above treated regions in amounts of 0.001 mg/m^3 when used at the rate of 6 kg/ha, whereas only traces were found when used at the rate of 2 kg/ha. The amount of DDVP in the leaves constituted 1.2-2.0 mg/kg, depending on the dosage used. DDVP was demonstrable in the soil on the day of treatment only when chlorophos was used at the rate of 6 kg/ha.

One day after treatment, the residual chlorophos in the superficial soil of vineyards doubled, as compared to the original level, whereas it decreased to one-half in the leaves. It should be

noted that there was some rain (about 5 mm) 1 h after spraying. It may be assumed that the higher concentration of chlorophos in the soil was due to it being washed off the surface of the grape vines.

The chlorophos content of grape leaves constituted hundredths of a milligram per kg and that of superficial soil, hundredths or thousandths of a milligram per kg 15 days after treatment. When used at the rate of 6 kg/ha, the level of air contamination was somewhat higher than with 2 kg/ha.

Chlorophos was demonstrable in the air over the region treated with it at the rate of 6 kg/ha for 10 days, whereas when used at the rate of 2 kg/ha no insecticide was demonstrable after 6 days, while DDVP level in the air constituted 0.003 mg/m^3.

DDVP, the metabolite of chlorophos, was demonstrable in all media for 6 days after treatment, i.e., when the chlorophos levels in leaves and soils were of the order of tenths to several milligrams per kg. With increase in dose rate of chlorophos, the DDVP content of grape vine objects also increased.

Thus, the level and time of retention of chlorophos and its metabolite, DDVP, in the environment were determined by several factors, among which the dose rate of the insecticide and climatic conditions were important.

The obtained data warrant the conclusion that when OPP are used in agriculture there is contamination of environmental objects (plants, soil and air), not only by the original products but by the products of their breakdown, rogor and DDVP. For this reason, when pursuing hygienic studies of the behavior of these pesticides in the environment, as well as in the course of sanitary inspections of levels of these agents in foods, soil and air, one must assay not only anthio and chlorophos, but their metabolites, rogor and DDVP.

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A METHOD OF ANALYSIS OF ECONOMIC LOSSES DUE TO ATMOSPHERIC POLLUTION OF POPULATED AREAS

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 46-49

[Article by K. P. Selyankina, candidate of biological sciences; Prof B. A. Katsnel'son and G. A. Bushuyeva, candidate of economic sciences, Sverdlovsk Scientific Research Institute of Industrial Hygiene and Occupational Diseases, submitted 25 Mar 75]

[Text] Our objective was primarily to compare the morbidity rate in groups of workers working under the same conditions at the same enterprise and presently living in the same populated area, which is subject to the significant influence of industrial atmospheric pollution, who differed only in that one of the groups consisted of individuals who were born there and spent their childhood and youth there ("local") and the second consisted of individuals who arrived there only at the age of 18 years, i.e., at the time they started to work ("newcomers").

We selected two plants as the objects of our study; they were in a small industrial town that has been exposed, for a long time, to the atmospheric waste from a nonferrous metallurgical enterprise, that pollutes the air with sulfur dioxide, certain other gaseous compounds of sulfur and dust of a complex chemical composition. We named an enterprise in the machine-building industry, with the usual working conditions for this industry, Plant No 1, and Plant No 2 referred to the main shops of the same metallurgical enterprise which is the source of atmospheric pollution. We formed groups of local and newcomer workers at each of these plants and obtained base material for comparative analysis of morbidity in these groups on the basis of a personal sorting of temporary disability certificates issued in 1970-1972. We did not include individuals who were over 37 years of age in 1970, who were born prior to opening of Plant No 2. The mean age of local workers (24.4 years for men and 26.3 years for women) was somewhat lower than of newcomers (26.5 and 28.8 years, respectively); to rule out the differences in morbidity indices that could be related to this, we standardized them according to age.

As can be seen in the Table, at Plant No 1, the standardized morbidity indices (in both number of cases and days per 100 people) were higher for both males

and females in the local groups than the corresponding newcomer indices. This consistent difference is due primarily to the differences in incidence of certain classes and forms of diseases. For males, this refers to diseases of respiratory organs, including pneumonia, diseases of the nervous system and sense organs (particularly, inflammatory diseases of the eyes), acute respiratory diseases, influenza, diseases of the esophagus, stomach and duodenum; for females, this refers to the same diseases, except the last mentioned, but in addition complications of pregnancy, parturition and the postpartum period, pathology of the mammary gland and genitalia. Of course, the ratio of morbidity indices of local workers to those of newcomers are even higher in many cases with reference to these groups of diagnoses than with regard to overall morbidity. For example, there was an average of 1.92 times more cases in 3 years of diseases of respiratory organs among local women and 2.2 times more days of absence; absenteeism due to acute repiratory diseases was 1.73 times higher and that due to influenza, 2.68 times higher. In spite of the few years covered by the study, the rather large size of the groups and consistent differences in the same feature render the findings quite reliable in most cases. An inverse ratio between groups was neither appreciable nor consistent for any of the classes or forms of disease.

Relation of indices, standardized according to age, of overall morbidity involving temporary disability for local workers to the same indices for newcomers

Plant	Year	Men		Women	
		cases	days	cases	days
№ 1	1970	1,62+	1,75	1,60	1,25
	1971	1,05	1,52	1,34	1,52
	1972	1,49+	1,29	1,70+	1,18
	3-year mean	1,34+	1,46+	1,40+	1,30+
№ 2	1970	0,80	0,80	—	—
	1971	1,56+	1,88	—	—
	1972	1,68+	2,08	—	—
	3-year mean	1,31+	1,57	—	—

Note: + refers to indices, the differences in which between local and newcomer workers are statistically significant ($P<0.05$). The significance of differences in number of sick cases in each year was determined according to V. A. Mosglyakova, and as the mean for 3 years by the method of combined samples (V. Yu. Urbakh). The significance of differences in days was evaluated only on the basis of mean indices as for small correlated samples (I. A. Plokhinskiy)

The table also shows that the ratio between newcomer and local males* at Plant No 2 coincides almost perfectly with the one at Plant No 1, according

*In view of the small number of women in the main shops of this enterprise, we processed only morbidity of male workers.

to the 3-year mean data. For this reason, the correlation between the plants is, in turn, the same, whether it is considered for local workers (2.04 times more cases and 2.15 times more days of disability at Plant No 2, as compared to Plant No 1) or newcomers (2.10 and 2 times more, respectively. In other words, the substantial difference in working conditions inherent in these plants is equally reflected in the morbidity rate of both groups, but whatever the working conditions the morbidity is higher among local workers than newcomers.

At the same time, we cannot fail to observe that at Plant No 2, unlike No 1, even over a 3-year period we found 1 year in which morbidity was 20% lower according to general indices among local workers than newcomers. Unlike Plant No 1, such an inconsistency in the main correlation between groups is demonstrable as well for all the diseases, due to which it is formed.

It may be assumed that the dissimilar average original condition at the time local and newcomer workers begin to work is manifested by the existence of a more marked difference in morbidity involving temporary disability at the plant where there are no marked deleterious industrial factors. Of course, it must be borne in mind that, among the newcomers, there are quite a few who were born and raised in rural areas, so that the difference in conditions under which they spent their childhood and those referable to local workers cannot be reduced solely to a difference in quality of atmospheric air. However, it is unquestionable that expressly the latter plays the most important role, particularly if we consider that the most substantial differences in indices were found for diseases of organs and systems that are usually prominent when the above-mentioned comparisons are made between morbidity of the child population of urban areas only, but with different levels of atmospheric pollution.

Against the background of more massive effects of the same pollutants (Plant No 2) related to the industrial environment, evidently the differences attributable to different living conditions in childhood are eroded to some extent but, on the whole, are of the same nature.

The actual economic forecast was based on an estimate, which proceeded from the following arbitrary premises: the children currently living will decrease in number in the future, in accordance to the longevity tables prepared for large industrial cities in similar geographic regions; the "additional" loss of work days referable to this group for the entire period of future work, related to conditions under which they spent their childhood, would correspond to the difference found between local and newcomer workers at an enterprise with satisfactory working conditions (Plant No 1); the economic loss corresponding to each day of absenteeism due to illness is also the same as we calculated for Plant No 1, according to all components of this loss.* It can be readily seen that, at least the first and last

*Disability payments, loss of production due to absence of a worker, mean cost of treatment.

of these prerequisites cause an inevitable underestimation of the projected losses: in the first place, due to the expected increase in future mean life expectancy and, in the second place, due to growth of productivity of labor, i.e., the "cost" per work day. However, even such a projection, known to be underestimated, indicates that about 809,600 rubles will be lost per 1000 children living in a region with atmospheric pollution from Plant No 2 over their future period of employment, as a result only of increased morbidity with temporary disability, which corresponds to about 19,300 rubles annually (as the mean for this period) for men and 22,700 rubles for women.

Another approach to evaluation of economic loss indicates that, even now, the annual economic losses related to temporary disability per local worker at Plant No 1 constituted 17-19 rubles more for men and 12-37 rubles more for women, as compared to the same indices for newcomers. It is evident from the views expressed that these figures can be considered one of the elements of economic detriment to society (at the present time and in the next few decades) caused by atmospheric pollution. Thus, they will be included as components of the economic effect, which can be obtained with effective hygienic control of atmospheric pollution.

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GAS CHROMATOGRAPHY USED TO MEASURE PHTHALATE PLASTICIZERS IN AIR WITH AN ELECTRON CAPTURE DETECTOR

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 59-62

[Article by M. T. Dmitriyev and V. A. Mishchikhin, Institute of General and Municipal Hygiene imeni A. N. Sysin, USSR Academy of Medical Sciences, Moscow, and Sanitary and Epidemiological Station of the 4th Main Administration Under the USSR Ministry of Health, Moscow, submitted 2 Sep 77]

[Text] Esters of dicarboxylic acids, including phthalate plasticizers, are used extensively for plastizing polymers. The levels thereof in air, in hygienically significant concentrations, have not been investigated enough, for which reason it is important to have an effective method of assaying them. Previously, L. V. Gortseva and L. I. Rapaport used an ionization-flame detector (IFD) for gas chromatic demonstration of dibutyl phthalate (DBP) and dioctyllphthalate (DOP) in air. Samples were collected in ethanol, 1 μℓ of which was put in the chromatograph. With a 30-ℓ air sample, the minimum demonstrable concentrations constituted 0.04 mg/m^3, which corresponds to analytic sensitivity of about 10 ng in the sample.

As we know, the sensitivity of an electron capture detector (ECD) is 10-100 times higher than IFD, depending on the properties of the compounds to be demonstrated (M. T. Dmitriyev and N. A. Kitrosskiy, 1966). They also include carbonyls with conjugate dual bonds (V. G. Berezkin; Bunting and Walker). Moreover, the ECD makes it possible to use solvents that have little sensitivity to this detector, which improves the characteristics of chromatograms and shortens the time required for analysis. We used the Tsvet-106 chromatograph with ECD for gas chromatography of DOP and DBP in air.

Figure 1 illustrates how the ECD is connected to the gas chromatograph. Extra-pure nitrogen passes from gas processing unit *1* concurrently into the high-temperature chamber with constant recombination rate *2*, which blows out the detector, and into gas chromatography column *3*. The carrier gas (nitrogen) is dispensed at the rate of 80 mℓ/min, and 120 mℓ/min nitrogen is used to blow out the detector. One should use 50-100% more nitrogen to blow out the detector than as carrier in the column. The sample to be analyzed is put in evaporator *4*, and after passing through column *3* and detector chamber *2*, it is diverted in ventilation channel *5*. The column and detector are in

the column thermostat *6* and detector thermostat *7*, respectively. The temperature of the detector is 250°C, and it should be 30-50°C higher than in the column. The evaporator temperature must be 10-20°C higher than the boiling point of the tested substance and, in our case, is 250°C. The electric signal from the chamber is delivered to portable detector unit *8*, which has a power pack *9*, and is amplified on low current gage *10*, which is equipped with an electronic potentiometer *11*. The sensitivity of the potentiometer amplifier is $10 \cdot 10^{-12}$ A with a 10 mV scale on the automatic potentiometer.

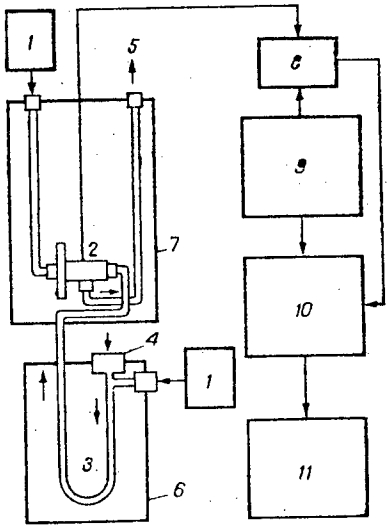

Figure 1.
Diagram of hook-up of electron capture detector to a gas chromatograph. Explanation is given in the text.

The substances were separated in a glass column, on an SE-30 silicone elastomer applied to the chromaton [?] in 0.4-0.6 mm granulations (5% of the solid carrier mass). The column is 1 m long, 5 mm in diamter, with a temperature of 215°C. The sample size is 1 μℓ, and the amount of demonstrable plasticiers is 1 ng. Typical chromatograms are illustrated in Figure 2. In the case of heptane, the solvent peak is 20-30% narrower, so that separation thereof from DBP is somewhat better. Consequently, the use of heptane results in more accurate assay of DBP, as compared to ethanol. DBP retention time is 56 s and that of DOP is 4 min and 45 s.

Samples (of 1 μℓ each) were added to heptane solution. In the recommended range of demonstration (0.5-20 ng), there is strict adherence to linear correlation between height of the peak and added sample. The sensitivity of analysis with the ECD constitutes 0.5 ng for DBP and 1 ng for DP, which is 10-20 times greater than demonstration of phthalates with the IFD.

Some difficulties arise in collecting sample, when using gas chromatography for demonstration of toxic substances in air. The highest concentrations can be obtained when samples are collected on solid sorbents or cooled

sample-collection tubes (M. T. Dmitriyev and N. A. Kitrosskiy). However, the use of solid sorbents requires meticulous preliminary testing of degree of adsorption and desorption, as well as heat stability of tested compounds at desorption temperature. Liquified gases (nitrogen or oxygen) or dry ice must be available for collecting samples with cooling, and this limits, to some extent, adoption of gas chromatography techniques. Samples of many substances can be collected in uncooled tubes, filled with a checker with stationary phase on a solid carrier (M. T. Dmitriyev and L. D. Pribytkov).

At the same time, it is also quite possible to collect samples in absorption instruments with a porous plate, generally used for subsequent photocolorimetry or spectrophotometry. Since, in this case, the sample of substance is distributed in a rather large volume of absorbent solution (up to 5 mℓ), most of the sample is not used subsequently for analysis, since it is not expedient to put samples of more than 1-5 mℓ in the chromatograph. However, if the high sensitivity of the chromatograph still permits demonstration of concentrations at the MPC [maximum permissible concentration] level, this flaw in the method of collection, of course, becomes insignificant. In addition, along with loss of sensitivity there is also the possibility of partial compensation thereof as a result of subsequent evaporation of the absorbent solution to a reliably measurable volume (of the order of 0.2 mℓ). At the same time, when air samples are collected in this manner, air oxygen, condensed water and carbon dioxide, as well as methane, light hydrocarbons or other substances with low melting point do not affect the chromatogram, and this is undoubtedly an important advantage of this method.

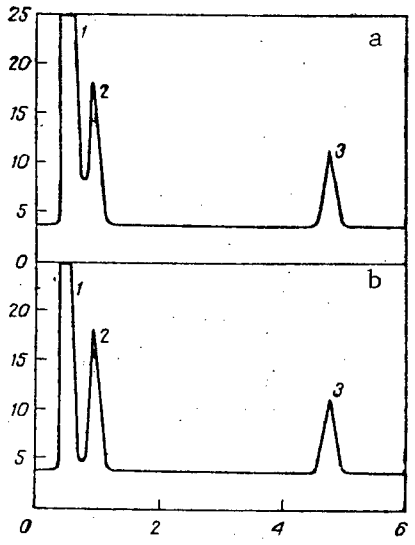

Figure 2.
Typical chromatograms of phthalate plasticizers with the use of ethanol (a) and heptane (b).

X-axis, recording time (min); y-axis, height of tracing (cm)

1) solvent
2) DBP
3) DOP

Important factors in all forms of collection are to reach full capture of substances during collection and minimum loss in subsequent operations, for example evaporation. To assay phthalate plasticizers, air samples are collected in two successively connected instruments with a Zaytsev porous

plate, filled with 3 mℓ heptane or ethyl alcohol. Collection rate is 0.5 ℓ/min for 20 min. The sample is then evaporated to 0.2 mℓ. Samples of 1 μℓ are put in the chromatograph. Minimum demonstrable concentrations constituted 0.01 mg/m^3 for DBP and 0.02 mg/m^3 for DOP. Consequently, the method we developed permits demonstration of phthalate plasticizers on the level of the MPC for atmospheric air (0.05 mg/m^3).

A test of the method with preset concentrations created by a graduating device revealed that fullness of detection of phthalates with both solvents is in excess of 95-98% with consideration of subsequent losses due to evaporation of samples. An analogous method for collecting DBP and DOP was used previously for assay thereof in air by means of thin-layer chromatography (S. Ye. Katayeva and V. I. Kofanov) and gas chromatography with an IFD (L. V. Gortseva and L. I. Rapaport).

Phthalate content of air is determined by using a simple equation:

$$C = \frac{M \cdot S_y}{V \cdot S}$$

where C is phthalate concentration (mg/m^3), M is the amount of the substance determined on a calibration chart (ng), S_y is the volume of absorbent solution after evaporation (mℓ), S is the volume of the sample put in the chromatograph (μℓ) and V is the volume of the air sample reduced to normal conditions (ℓ).

With the recommended S_y = 0.2 mℓ, V = 10 ℓ and S = 1 μℓ, C = 0.02 M.

Thus, the use of the ECD for demonstration of phthalate plasticizers increased the sensitivity of analysis (by 10-20 times), reduced the size of the air sample (to one-third) and time required for chromatographic analysis as a result of lower (one-third to one-quarter) sensitivity of ECD to the solvent. The developed method has been used in hygienic studies of polymers, plasticized DOP and DBP.

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SEPARATE ASSAYS OF DIPHENYLISOBUTYLPHENYL PHOSPHATE AND PHENOL WHEN EXAMINING POLYMERS

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 62-63

[Article by S. K. Osokina, Moscow Scientific Research Institute of Hygiene imeni F. F. Erisman, submitted 8 Aug 77]

[Text] At the present time, an organophosphorus compound, diphenylisobutylphenyl phosphate, is added in polyethylene products. In the course of production and use of polymers based on polyethylene, with addition of the above-mentioned antipyren [fireproofing compound], compounds such as diphenylisobutylphenyl phosphate [DBIBPP] and phenol, which is a product of decomposition of the latter, may migrate into contact media.

We found no descriptions in the available literature of methods for separate assays of small amounts of DPIBPP and phenol when both are present. We know of a method of assaying phenol, which is based on the capacity of alkaline solutions thereof to absorb ultraviolet light in the range of 200-305 nm waves, with maximum absorption at 235 nm, followed by photometric determination on a graduated graph, plotted on the basis of measurement of standard scales in the optical density -- concentration coordinates (M. D. Manita).

The spectrophotometric method, which makes rapid determination possible, involves simple operations and can be used over a wide range of concentrations of toxic compounds, has also been used for separate quantitative assays of DPIBPP and phenol in hygienic studies. Since spectrophotometry is suitable for differential assay of components in a mixture, if the substances have individual light-absorption properties, at first one obtains the absorption spectra of the tested substances in the range of 220-350 nm.

In view of the fact that the absorption spectra of DPIBPP and phenol are indicative of possible identification of these compounds, separate assays thereof have been made by the method of Firordt, which is widely used in the case of mixtures of two ingredients, when light absorption of each is known. A. Gillem and Ye. Shtern, who reported on this, cite some calculation formulas to measure the concentrations of substances in a tested solution.

Adhering to the law for spectrophotometric analysis, working wavelengths for separate assays of DPIBPP and phenol were so selected as to have the intensity of light absorption of these two compounds very different at one wavelength (270 nm) and similar at another (255 nm).

It was observed that, at these wavelengths, there is a proportionate relationship between optical density and concentration of the tested substances.

On the basis of established indices of optical density of solutions of DPIBPP and phenol, as well as mixtures thereof, at the above wavelengths, the formula for the system of two equations of Firordt used under our conditions would have the following appearance:

$$X = \frac{(D_{sam}^{255} \cdot D_{dpp}^{270}) - (D_{sam}^{270} \cdot D_{dpp}^{255})}{(D_{p}^{255} \cdot D_{dpp}^{270}) - (D_{sam}^{270} \cdot D_{dpp}^{255})}$$

$$Y = \frac{(D_{p}^{255} \cdot D_{sam}^{270}) - D_{sam}^{255} \cdot D_{p}^{270})}{(D_{p}^{255} \cdot D_{dpp}^{270}) - (D_{p}^{270} \cdot D_{dpp}^{255})}$$

where X is the concentration of phenol, Y is the concentration of DPIBPP, D_{sam} is the optical density of the mixture of DPIBPP (dpp) and phenol (p), 255 and 270 are wavelengths (in nm) [sam stands for sample].

We checked the validity of using the calculation method of Firordt for our studies on solutions with known amounts of DPIBPP and phenol.

The results of the experiments revealed that the error factor of the method does not exceed 12.5% and sensitivity constitutes 1 μg/mℓ.

Spectrophotometric assays of these compounds were used to test polymers manufactured on the basis of polyethylene with addition of DPIBPP as antipyren.

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A METHOD OF LOWERING TOXICITY OF EXHAUST FROM INTERNAL COMBUSTION ENGINES

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 86-87

[Article by Endre Pattantyush-Kh., candidate of engineering sciences, Hungarian People's Republic, Budapest, submitted 5 May 77]

[Text] In modern internal combustion engines, the process of combustion of liquid fuel depends on the extent to which it is atomized and homogeneity of the air and fuel mixture. In recent years, in engines with spark ignition, increasing use has been made of the practice of directing fuel directly in the cylinder, instead of forming a mixture outside, by means of a carburetor.

Such a mixing method results in two opposite tendencies: on the one hand, the particles of fuel coming out of the nozzle are reduced in mass as a result of evaporation and gas discharge and, on the other hand, they collide and adhere to one another in the turbulent flux, which increases their mass. The latter effect retards the fuel combustion process, which results in an increased amount of products of incomplete combustion in the exhaust of engines.

The principle of the method we recommend for lowering the toxicity of exhaust from internal combustion engines consists of exposing the fuel to ionizing radiation as it comes out of the nozzle, under the influence of which which the particles of fluid receive a homogeneous electric charge and, by virtue of repulsion, they break down into smaller drops. This enhances the combustion process and lowers the toxicity of exhaust gases (Pattantyush-Kh.; Feynman et al.).

It is known that the molecular structures of some compounds change under the influence of ionizing radiation (A. V. Zimin et al.; I. V. Vereshchinskiy and A. K. Pikayev; Swallow; Bolt and Carrol; Rexer and Wuckel).

As for the process of fuel combustion, it is manifested by the production of combustible gases (hydrogen, methane and others) from the hydrocarbon fuel and decomposition of multinuclear hydrocarbons. This is beneficial, both from the standpoint of heat engineering and protection of the environment from pollution by the toxic exhaust from internal combustion engines.

A rather important factor is that this precludes the need for using lead compounds or other antiknock additives.

Special studies are required on the question of the economy of this method of lowering the toxicity of engine exhaust. Evidently, additional expenditures are required to install a source of ionizing radiation on a motor vehicle; however, one should also take into consideration the economic effect from ameliorating the environment.

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POLLUTION OF ATMOSPHERIC AIR OF KAZAN' BY BENZ(A)PYRENE IN THE RANGE OF A PETROCHEMICAL PLANT

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 97-99

[Article by F. F. Dautov, Kazan' Medical Institute imeni S. V. Kurashov, submitted 30 Jun 77]

[Text] Our objective was to determine the sources of air pollution by benz(a)pyrene (BP) at outdoor facilities and between shops, as well as to measure the level thereof in the atmospheric air of a residential block. A control point was selected far from industrial enterprises and major highways.

The industries under study can be sources of pollution of atmospheric air by hydrocarbons, ethylene oxide, acetone, phenol, isopropylbenzene and others, due to emission of gases through leaks in equipment situated out of doors and indoors, accidental dumping and numerous "vozdushki" [probably typo for vents]. For this reason, several engineering measures were implemented in the shops: the hatches of equipment have airtight lids, pump oil gaskets were replaced with end gaskets, a cyclone was installed to reduce discharge of liquid hydrocarbons, a new fractionating column [dephlegmator] was installed on the column of commercial acetone, vortical heat exchangers were installed to trap fumes of hydroperoxide and isopropylbenzene. All granulation equipment is equipped with cyclones, there are bag-type filters on the bunkers for production of carbon black paste and vacuum filters on the bunkers of the polyethylene processing shop in order to lower air pollution by polyethylene dust. Laboratory monitoring of efficacy of operation of the gas and dust trapping devices revealed that their technical efficiency is consistent with the projected level.

The steps taken at this plant resulted in a reduction of pollution of atmospheric air in the residential block by toxic substances (M. M. Gimadeyev, F. F. Dautov).

In our study of air pollution by BP, we were guided by the methodological instructions on collecting samples from environmental objects and preparing them for analysis to demonstrate carcinogenic polycyclic hydrocarbons (1972). Samples were taken using a device designed by M. M. Gimadeyev and F. F.

Dautov. The amount of air pumped through the device constituted 225-250 m^3/h. We used FPP-15 synthetic material as a filter. The spectral fluorescence method was used to assay BP. Using this device, 295 air samples (at least 4 from each point) were collected and analyzed in 1974-1976. We took samples from different buildings in the course of the technological process, near equipment and on the territory of the shops to detect sources of BP production.

Table 1. BP content in industrial air

Process	Site where samples were collected	Numb of samp.	BP content, µg/100 m^3		
			minimum	maximum	most often found
Gas separation	Indoors	30	0,011	0,378	0,018—0,257
	Outdoor install.	23	0,062	0.98	0,063—0,75
	Between shops	24	0,035	0,394	0.058—0,147
Ethylene oxide production	Indoors	73	0.012	0,398	0.045—0,216
	Outdoor install.	12	0,033	0.435	0,044—0,125
	Between shops	8	0,004	0,093	0.009—0,061
High-pressure polyethylene	Indoors	33	0,001	0,385	0.012—0,157
	Between shops	9	0,001	0,03	0,008—0,01
Organic peroxides	Indoors	44	0	0,276	0,002—0,128
	Between shops	5	0	0,011	0,001—0.01
Phenol and acetone	Indoors	21	0,022	0,334	0,027—0,164
	Outdoor install.	8	0,03	0,133	0,053—0,112
	Between shops	5	0,029	0,157	0,044—0,126

Note: The maximum permissible concentration of BP indoors is 15 µg/100 m^3, and in the basin between shops, 30% of the MPC for work places.

The results of testing aspiration samples are submitted in Table 1. It should be mentioned that BP was found in 99% of the samples, but its levels were always below the MPC [maximum permissible concentration]. Maximum BP pollution was found near the outdoor equipment and in the air of gas-separation shops. The lowest BP level was demonstrated in the inter-shop air basin where organic peroxides are produced.

Testing of air samples collected near equipment revealed that the outdoor installations (pyrolysis furnaces) for gas separation and furnaces used to heat ethylene oxide production were the greatest sources of pollution of atmospheric air. In the atmospheric air of the residential block (1000 m away from the plant), BP was demonstrable in concentrations of 0.0007-0.0780 µg/100 m^3.

It must be indicated that there was less fluctuation of BP level at a distance of 500 m from the plant (0.006-0.042 µg/100 m^3) than at 1000 m.

The wind direction has a significant influence on the extent and nature of BP pollution of atmospheric air around plants. The concentration of BP was always higher (0.013-0.3 μg/100 m^3) on the leeward side than on the windward side (0.009-0.07 μg/100 m^3).

At the control point, BP content ranged from 0.0003 to 0.0050 μg/100 m^3 air, i.e., it was 15-18 times lower than in the residential block.

We also studied the extent of atmospheric air pollution by BP by analyzing 39 samples of snow. We collected snow in areas with a uniform layer thereof when possible, where the snow cover was not exposed to gusts of wind or build-up of drifts. The results of assaying BP levels in samples of snow are listed in Table 2.

Table 2. BP content of samples of snow collected near a petrochemical plant

Origin of samples	Number of samples	Range of BP levels over a 132-day period, μg/m^2
Territory of plant	23	0.4-129
At following distance from plant:		
500 m	4	0.2-12.4
1000 m	4	1.3-43.0
1500 m	4	1.5-13.9
Control regions	4	0.9-6.9

As can be seen in Table 2, maximum BP levels in samples of snow were observed at a distance of 1000 m from the plant, which is consistent with the results of testing samples of atmospheric air.

Thus, the petrochemical plant we studied is a source of BP pollution in the atmospheric air of the residential block. It is not present by itself in the territory of plants and urban atmospheric air, but demonstrable together with toxic substances. We failed to demonstrate a direct correlation between concentration of toxic substances and BP level.

After analyzing the causes of atmospheric air pollution by BP, we concluded that it occurs in connection with petrochemical plants in the course of heating the gas fractions in furnaces and tubular reactors, as well as circulation of products in pipes and equipment. Several ameliorative steps were taken at the plant on the basis of the obtained data. At the present time, technological waste discharged into atmospheric air is submitted to treatment, with the use of neutralizer-scrubbers, washer scrubbers, reverse condensers, silica gel filters and foam gas purifiers. Because of the corrosive properties of some products, the main equipment is made of rust-proof materials (stainless steel, aluminum, enamel and plastic coatings, etc.), which reduces pollution of air by toxic substances. In addition,

several pumps, shut-off fittings and pipes are lined with fluoroplastic, polyethylene, etc. Construction is being completed on a unit to collect and utilize gas waste at heat and electric power plants.

In view of the fact that urban atmospheric air is polluted by BP, the program of studies of sanitation laboratories of petrochemical plants must include assays thereof in the areas affected by the plants.

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REVISION OF MAXIMUM PERMISSIBLE CONCENTRATION OF ALUMINUM IN WATER

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 101-103

[Article by L. Ya. Vasyukovich, candidate of biological sciences, T. G. Lamentova, L. F. Astakhova and I. N. Konstantinova, Institute of General and Municipal Hygiene imeni A. N. Sysin, USSR Academy of Medical Sciences, Moscow, submitted 4 May 77]

[Text] The maximum permissible concentration (MPC) of aluminum in water has been set at 0.5 mg/ℓ, according to the organoleptic index of deleteriousness, the index of cloudiness. This standard was scientifically substantiated by A. A. Petina and M. I. Nekipelov. In the past few years, it was established that, in concentrations of 20 to 1.5 g/kg, aluminum disrupts the metabolism of phosphorus compounds (Ondreicka; Waldron-Edward et al.; Bailay) and induces histological changes in brain tissue cells (Crapper); increased accumulation of aluminum in the testicles was also reported (Ondreicka). However, the effect of aluminum on reproductive functions was not taken into consideration in setting the MPC thereof in water, nor has its effect on conditioned reflex activity investigated, so that it was necessary to test the reliability of the previously set standard.

The biological effects of aluminum were studied in brief and chronic experiments. Aluminum-potash alum was administered intragastrically, in doses of 50, 17 and 6 mg/kg (estimated for the aluminum ion) to rats and guinea pigs and in doses of 27, 9 and 3 mg/kg to rabbits. By the end of the experiment, we demonstrated a substantial decrease in blood serum alkaline phosphatase activity, change in levels of ATP and its derivatives in blood under the influence of the highest of the tested doses. The minimum effective dose of aluminum, according to effects on these parameters, was set at the level of 17 mg/kg for rats and guinea pigs and 9 mg/kg for rabbits. This applies only to the change in activity of alkaline phosphatase in blood serum; rats, guinea pigs and rabbits are equally sensitive to aluminum according to parameters of acute toxicity.

The study of spermatozoal function in the animals revealed that a distinct gonatotoxic effect was manifested only in doses of 27-50 mg/kg aluminum. However, one of the tested parameters, motility of spermatozoa, was somewhat lower in guinea pigs and albino rats given 17 mg/kg aluminum. Thus, a general and gonadotoxi effect was demonstrable under the influence of the same doses of aluminum.

Table 1. Rat blood serum phosphatase activity under the influence of aluminum in a chronic experiment (M±m)

Dose, mg/kg	Phosphatase activity (per 100 units FEK)* after giving aluminum-potash alum					
	1st month	2d month	3d month	4th month	5th month	6th month
Control	16,2±1,2	14,2±1,1	17,1±0,4	18,0±0,7	15,8±1,1	14,0±0,9
0,025	16,8±1,8	15,3±0,6	18,0±1,0	16,7±0,6	16,2±1,3	16,0±1,2
0,25	12,8±0,5 $P<0,05$	13,7±1,0	15,5±1,3	19,1±0,9	15,7±0,9	15,6±1,6
2,5	11,9±0,6 $P<0,05$	10,0±0,8 $P<0,01$	13,2±0,9 $P<0,01$	19,6±2,3	13,3±1,3	8,9±0,2 $P<0,01$

*FEK extinction--expansion unknown.

Table 2. Higher nervous activity in rats after chronic enteral aluminum poisoning (M±m)

Dose of aluminum, mg/kg	Formation of conditioned reflex (number of combinations)		Latency period, s	Uncondition. reflex, mA	Conditioned reflex, mA	Extinction of reflex, mA	Restoration of reflex	
	appearing	fixed					appearance	fixed
Control	11,7±0,97	26,3±3,56	0,81±0,22	54,0±7,18	36,0±4,63	44,0±2,02	8,0±1,07	11,0±2,05
0.025	12,9±1,2	28,0±2,7	0,8±0,31	52,0±2,8	34,0±3,16	45,0±3,4	8,0±1,0	12,0±2,1
0.25	21±8,6	35±7,5	0,85±0,1	49±8,9	25±0,97	40±3,69	5,0±0,74	8±3,15
2.5	40,7±3,4 $P<0,05$	61,3±6,71 $P<0,05$	1,17±0 $P<0,05$	47,0±9,5	31,0±7,69	16,0±9,67 $P<0,05$	7,0±0,24	19,0±4,4

We tested the effects of aluminum in doses of 2.5, 0.25 and 0.025 mg/kg, calculated per ion of aluminum, in a chronic 6-month sanitary and toxicological experiment on albino rats. We demonstrated changes in blood serum phosphatase activity (Table 1) and impairment of conditioned reflex activity (Table 2) in rats given 2.5 mg/kg aluminum. The nature of the behavioral reactions, recorded on the Swedish Animex instrument, was impaired only against the background of slow movements, unrelated to movements about the chamber (33.9±3.8 arbitrary units in the control; 20.7±4.0 arbitrary units in the experiment, $P<0.05$). Histological analysis revealed that, with a dosage of 2.5 mg/kg, there is venous plethora of the liver and activation of reticuloendothelial system cells; we encountered hemorrhagic areas and swollen hepatocytes (signs of granular dystrophy) in many fields of vision, with pyknosis and lysis of nuclei, as well as increase in lymphoid-histiocytic infiltrates, increased basophilia of hepatocytes in peripheral parts of the lobes. There were more RNA granules in the peripheral parts of the hepatic lobes of experimental animals. While glycogen was diffusely and uniformly distributed in the liver of control animals, there was a distinct decrease therein after a dosage of 2.5 mg/kg, with occasional disappearance thereof in the middle of the lobes; here and there we succeeded in detecting so-called "mosaic" distribution of glycogen (alternation of cells without glycogen and those filled with it).

We were unable to demonstrate a difference in activity of succinate dehydrogenase. There was increased activity of hydrolytic enzymes in large vessels and sinuses. Examination of the testicles revealed a distinct gonatotoxic effect of aluminum, manifested by subtotal gonadal lesion. Pathological forms of spermatogenic epithelium (Maksimov cells) were encountered in the depleted tubules and there was significant proliferation of interstitial cells (Leydig cells). We should also mention sclerosing of the tunica albuginea of the testes. Disturbances referable to plastic and energy metabolism, demonstrable in rat testes, were totally consistent with the structural changes. There was a sharp reduction of RNA content in the parenchyma of the gland, as well as depressed activity of oxidative and hydrolytic enzymes. Against this background, some of the intact tubules were noteworthy for increased RNA content and increased activity of oxidative enzymes. These findings were confirmed by compensatory changes in intact regions.

With reference to interstitial tissue, there was development of histochemical changes in the opposite direction: increased activity of oxidative enzymes in Leydig's cells, elevated ATPase level in the basement membranes of seminiferous tubules and walls of blood vessels. Thus, against the background of insignificant [changes] parenchymatous organs, we demonstrated a distinct gonadotoxic effect of aluminum when administered for a long time. In addition to this metal's direct effect on the spermatogenic epithelium, we cannot rule out the possibility of its mediated effect through the vascular system. Functional tests also confirm the gonatotoxic effect of aluminum, manifested by a change in number of spermatozoa as determined on a TsG-2 instrument (208±10 arbitrary units in the control, 154±17 arbitrary

units in the experiment, $P<0.05$) and their motility time (6.0±0.3 h in the control and 4.08±0.5 h in the experiment; $P<0.05$).

After giving a dose of 0.25 mg/kg, there were changes in alkaline phosphatase activity only in the first month of intake, followed by normalization to the control level. Since there were no changes in the other tests, to define the significance of this dosage of aluminum as the threshold level, we made a biochemical study of its effect on energy metabolism of the liver and testes at the end of the chronic experiment. We demonstrated a 42% decline of inorganic phosphate utilization ($P<0.01$) in the process of oxidative phosphorylation. However, this decline did not lead to impairment of the process of conjugation of respiration and phosphorulation: the coefficient of phosphorylation (P/O) did not change. The adenine nucleotide content of the rat testes did not differ from normal. Consequently, the dose of 0.25 mg/kg aluminum can be assessed as close to the threshold level. There were no changes in indices used in the study of the testes with a dosage of 0.025 mg/kg.

Thus, a concentration of 50 mg/ℓ, or 2.5 mg/kg, aluminum in water should be considered hazardous to public health and capable of inducing a marked toxic effect. The concentration of 5 mg/ℓ (0.25 mg/kg) can be considered as a threshold, while 0.5 mg/ℓ (0.025 mg/kg) does not affect the organism.

Conclusion

To sum up the obtained data, we do not consider it necessary to set a new MPC for aluminum (0.5 mg/ℓ) in water, but it is expedient to alter the index of deleteriousness and set the aluminum standard for water according to the sanitary and toxicological index.

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NEW BOOK DEALS WITH SANITARY PROTECTION OF URBAN ATMOSPHERIC AIR

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 p 120

[Review by V. Ye. Polyak, candidate of medical sciences, of the book "Sanitarnaya okhrana atmosfernogo vozdukha gorodov" by R. S. Gil'denskiol'd, M. K. Nedogibchenko, M. A. Pinigin and Yu. G. Fel'dman, Izdatel'stvo Meditsina, Moscow, 1976, 168 pages]

[Text] This book deals with an extremely timely problem of modern cities, that of protecting atmospheric air against pollution by the waste of industrial enterprises and exhaust from motor vehicles.

The first chapter analyzes the present status of this problem and contains information about legislation dealing with protection of atmospheric air.

The second chapter discusses some of the scientific problems of atmospheric air, including principles to apply in setting permissible levels of atmospheric pollutants, methods used to substantiate MPC [maximum permissible concentrations], and a comparison is made of the MPC set for the USSR and several foreign countries. The authors demonstrate a correlation between three factors: dosage (concentration), time and toxic effect, which are related to a general quantitative evaluation of interaction of toxic substances with the organism; there is discussion of the cumulative effects of chemical compounds; nomograms are proposed for evaluating the extent of pollution of atmospheric air as related to the degree to which the MPC is exceeded, hazard category of substances and extent of averaging of concentrations, as well as a method for overall evaluation of the hazard of a pollutant.

The third chapter deals with questions of preventive sanitary surveillance, which should be taken into consideration in expert evaluation of drafts at hygienic institutes and examination of drafts at sanitary and epidemiological stations. The authors call attention to listing of atmospheric pollutants when planning power plants and enterprises referable to various branches of industry, as well as consideration of many factors that affect the estimates of expected atmospheric pollution, which are made when designing a specific industrial enterprise. Special attention is given to the relative location of populated centers and industrial enterprises, as well as organization of sanitary protection belts around the latter.

The short fifth chapter deals with the main questions that must be taken into consideration in expert hygienic evaluation of plans for laying out and constructing cities with reference to protection of atmospheric air. The author of this chapter validly mentions the unstable operation of dust- and gas-trapping installations, the efficiency index of which is often below specifications, which results in an increased discharge of toxic substances into the atmosphere.

The sixth chapter is concerned with protection of the urban air basin from pollution by exhaust fumes from motor vehicles.

The seventh chapter discusses pollution of atmospheric air by waste from different branches of industry: chemical, metallurgical, oil-refining and petrochemical, construction materials, as well as heating and power plants; measures are offered to lower pollution of atmospheric air due to this type of waste.

Of considerable value are the practical orientation of the book, especially with regard to organization and implementation of preventive sanitary supervision at all stages, as well as confirmation of the presented material with specific examples from expert evaluation of plans and experience in supervising existing enterprises. The book is well illustrated. There is a bibliography at the end. Unfortunately, of the 70 cited literature sources, only 12 are listed.

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NEW BOOK DESCRIBES EXPRESS METHODS OF DETECTING TOXIC CHEMICALS IN ENVIRONMENTAL OBJECTS

Moscow GIGIYENA I SANITARIYA in Russian No 5, 1978 pp 120-121

[Review by Prof M. A. Klisenko of the book "Ekspressnyye metody obnaruzheniya yadokhimikatov v ob"yektakh vneshney sredy" by A. K. Koshcheyev, S. D. Livshits and V. F. Petrov, Perm', 1976, 225 pages]

[Text] In a previously published review (G. M. Makhkamov and I. G. Sivkov),* the contents and some of the qualities of this monograph were described. However, it is difficult to agree with the reviewers that it is a valuable publication for specialists in the field of analysis of residual amounts of pesticides. Not only is the book not useful, it is harmful, since most of the formulas recommended in it are obsolete and their sensitivity does not meet the requirements made of methods for checking pesticide residues.

For example, the permissible residual quantities (PRQ) of DDT in various food products (in mg/kg) constitute 0.1-0.2 for vegetables and grains, 0.003 for milk and dairy products and 0.1 for butter and fat.

The range of demonstration of DDT using the methods recommended by the authors is 0.025 to 16 mg, i.e., the product can be detected if its level exceeds the PRQ by 2000-5000 times.

The authors believe that rapid readings can be obtained by discarding such analytic stages as purification of the extract; however, they are in error. Indeed, purification of extract is the longest and most laborious operation, but if this stage of analysis is eliminated the toxic chemical under study cannot be detected due to cloudiness and atypical color of solutions of samples. This applies not only to analysis of organochlorine (pp 13, 17, 18, 25), but other pesticides (pp 39, 43, 47, 67).

The technique for demonstration of hexachlorobutadiene in water (p 24) is not selective in the presence of chlorine-containing compounds.

*GIG. I SAN. [Hygiene and Sanitation], No 1, 1977, p 122.

The methods for detection of aldrin, dieldrin and endrin (pp 25, 26) may be used only to identify the active principle in technical-grade products. the use of which was banned long ago in the USSR and none is stocked in warehouses.

The method for detection of organophosphorus toxic chemicals on the basis of the acetaldehyde formed from the epoxy group (p 33) is also arbitrary and suitable only for analysis of technical-grade products.

One can detect organophosphorus toxic chemicals in feed and water (p 36) only if the levels thereof are 5 or more times higher than permissible.

In the method for detection of chlorophos in washings from foods of vegetable origin (p 37) it is erroneously indicated that presence of pollutants in the examined objects does not hinder detection of this product.

The method for detection of malathion (p 45) is intended for analysis of the technical-grade product.

The method for detecting parathion in vegetable oils can be used if the toxic agent is present in amounts of over 33 mg/ℓ (none is allowed in food products) and it is not specific. Moreover, the use of this product has already been banned in the USSR.

Experimental verification of the methods for detection of sevin in water and vegetable products (p 67) revealed that fluorescence of the analyzed solutions is also observed in control samples.

The method for detection of 2,4D is not selective (p 80).

Most of the methods covered in the monograph are described in such a way that they cannot be reproduced, since there is no information on preparing samples for analysis, extracting agents used, extraction time, etc.

In conclusion, it should be mentioned that for 15 years there has been a team of experts dealing with analysis of residual pesticides in foods and the environment that has been working well in the USSR under the State Commission for Chemical Agents Used to Control Pests and Diseases of Plants and Weeds, of the USSR Ministry of Agriculture. In these years. this team has tested and recommended for official approval by the USSR Ministry of Health more than 100 methods for detecting microquantities of pesticides in foods, water, soil and feed.

The methods that are approved by the USSR Ministry of Health are published regularly in collections entitled "Metody opredeleniya mikrokolichestv pestitsidov v produktakh pitaniya, kormakh i vneshney srede" [Methods of Detecting Trace Quantities of Pesticides in Foods, Feed and the Environment].

However, there is still a need for express methods of analysis, but unfortunately the book being reviewed did not fill this gap.

Obsolete data (p 85) and repetition (pp 131 and 164) are encountered in the book.

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